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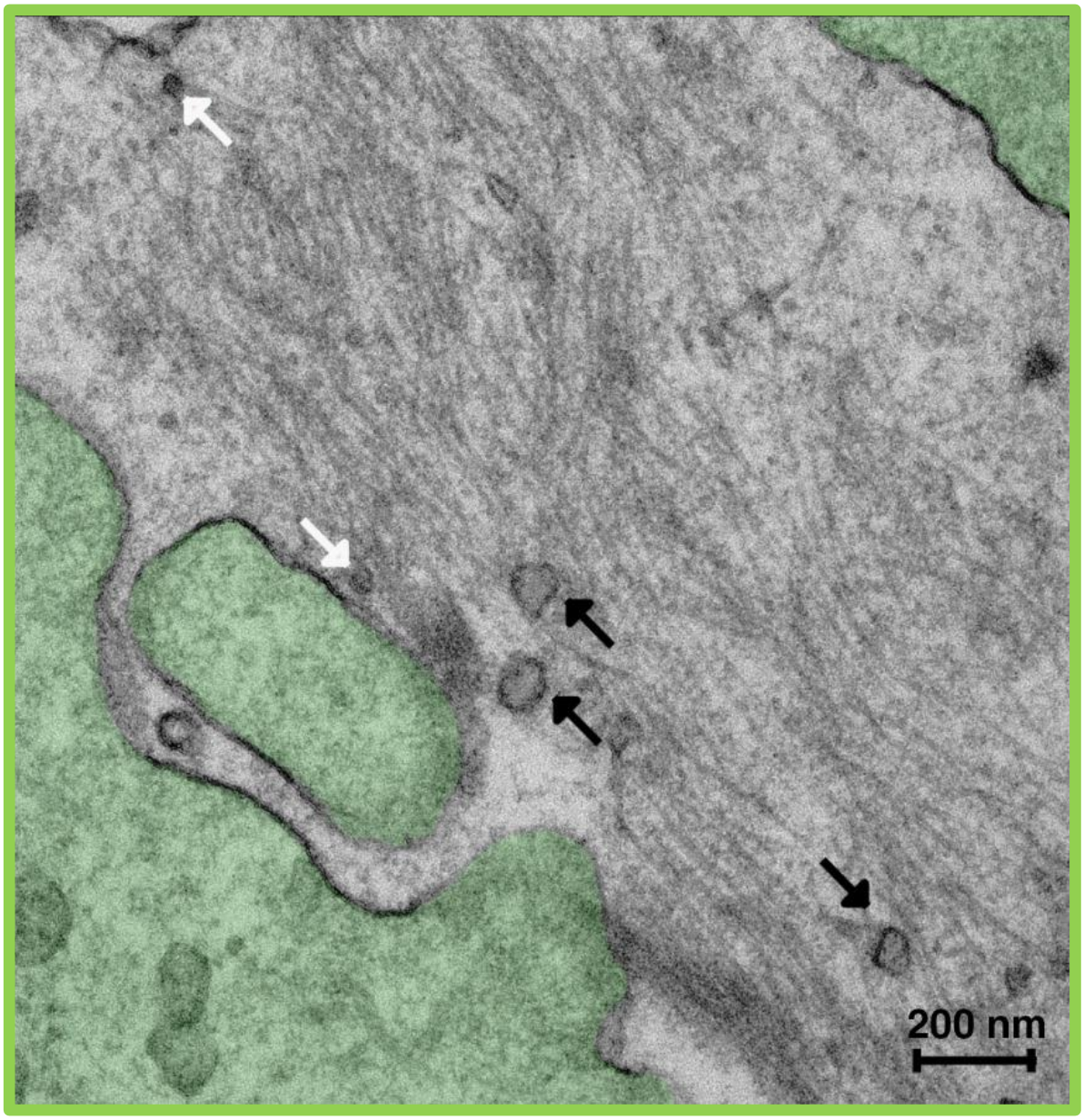

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WHAT'S NEW IN SCIENCE?

WHAT'S NEW FROM IEO RESEARCHERS?

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What's new in science?

Dissolvere gli aggregati di cellule tumorali circolanti per ridurre il potenziale metastatico delle cellule tumorali usando un farmaco per le patologie cardiache.

Nel primo studio clinico, gli autori hanno mostrato che la somministrazione di digossina, un farmaco comunemente utilizzato per il trattamento di condizioni cardiache lievi/moderate, in pazienti con tumore al seno metastatico, dissolve parzialmente gli aggregati di cellule tumorali circolanti nel sangue, riducendo il loro potenziale metastatico e possibilmente influenzando il decorso della malattia.

La malattia metastatica è la causa principale di decessi associati al tumore al seno; eppure, ad oggi non esistono farmaci specifici per le metastasi. Le metastasi hanno origine dalle cellule tumorali che lasciano il tumore primario, invadono il tessuto circostante, migrano nei vasi sanguigni e, attraverso il flusso sanguigno, raggiungono gli organi distanti dove danno origine a nuovi tumori: le metastasi. Nel flusso sanguigno, le cellule tumorali circolanti (CTC) "viaggiano" soprattutto aggregate in gruppi (i cosiddetti cluster di CTC). I cluster di CTC hanno una maggiore probabilità di dare origine a metastasi una volta raggiunto l'organo distante rispetto alle singole CTC. Nei pazienti con tumore al seno (e in altri tipi di tumore), la presenza di CTC nel sangue è stata associata con la presenza di metastasi e una minore sopravvivenza dei pazienti.

Studi precedenti in modelli preclinici in vivo hanno mostrato che la somministrazione di farmaci che dissolvono i cluster di CTC, come l'inibitore della ATPasi Na/K, sopprime le metastasi.

Scoperta principale. Un articolo recente riporta i risultati del primo studio clinico che ha valutato, in pazienti con tumore al seno metastatico, l'effetto sulla distruzione dei cluster di CTC della somministrazione di digossina, un farmaco comunemente utilizzato per il trattamento di condizioni cardiache lievi/moderate.

Dettagli. Gli autori hanno valutato l'effetto della somministrazione, in pazienti con tumore al seno, di digossina, a dosi sicure e ben tollerate (0.125-0.250 mg al giorno), sulla dissoluzione dei cluster di CTC e sul numero di cluster di CTC nel sangue, così come sulla cinetica della dissoluzione dei cluster e la relazione dose-risposta.

La digossina

La digossina è un farmaco approvato da FDA per il trattamento delle condizioni cardiache lievi/moderate. Sebbene, dopo la sua approvazione nel 1954, il suo utilizzo sia stato ampiamente sostituito (per il controllo del battito cardiaco) da beta bloccanti e inibitori dei canali di calcio, è tipicamente impiegato nel caso in cui altri farmaci risultino inefficaci, rallentando il battito cardiaco. Solitamente somministrato per via endovenosa, le iniezioni intramuscolari sono meno comuni. Rischi di tossicità noti sono associati con aritmie cardiache fatali, ovvero battito cardiaco irregolare (incidenza stimata tra 0.8% e 4% dei pazienti in terapia stabile con digossina) e aumentano con l'aumentare della concentrazione nel sangue del farmaco (>2ng/mL).

Le CTC isolate dai campioni di sangue di pazienti con tumore al seno trattate con digossina e pazienti di controllo non trattate con digossina sono state analizzate considerando la dimensione, il numero e il "tipo" di cluster (ovvero, cluster di CTC contenenti solo cellule tumorali (omotipici) o contenenti sia cellule tumorali che immunitarie (eterotipici)). Innanzi tutto, mentre i cluster di CTC delle pazienti trattate con digossina avevano dimensioni ridotte, non si osservava alcuna riduzione nelle dimensioni dei cluster in pazienti di controllo non trattate con digossina. Inoltre, confrontando la dimensione dei cluster prima e dopo trattamento con digossina hanno evidenziato una significativa riduzione della dimensione dei cluster dopo digossina. Concentrazioni più elevate di digossina nel sangue apparivano anche correlate con la ridotta dimensione dei cluster, sebbene la correlazione non fosse statisticamente significativa. Non hanno osservato eventi avversi associati alla somministrazione di digossina.

In modelli preclinici in vivo, hanno osservato una correlazione tra la ridotta dimensione dei cluster indotta da digossina e l'esito della malattia, con cluster di CTC più grandi dotati di maggiore potenziale metastatico (ovvero la capacità metastatica delle CTC era una funzione della dimensione dei cluster).

Infine, la caratterizzazione trascrittomica delle CTC isolate da pazienti, prelevate in momenti diversi, prima e dopo digossina (tre campioni raccolti prima della somministrazione di digossina e un campione raccolto dopo digossina) ha rivelato un diverso livello di espressione di 708 geni in seguito al trattamento (la maggior parte di questi geni era down-regolata dopo il trattamento). I geni diversamente espressi erano coinvolti nel ciclo cellulare e nell'adesione cellula-cellula, confermando dati precedenti che riportavano la perdita dell'adesione cellula-cellula e l'interferenza con il ciclo cellulare in seguito ad inibizione dell'ATPasi sodio/potassio.

Conclusioni. Studi precedenti hanno mostrato che la presenza di cluster di CTC nel sangue è associata con le metastasi e una scarsa prognosi dei pazienti. In questo lavoro, gli autori hanno scoperto che in pazienti con tumore al seno metastatico, che hanno manifestato recidiva dopo il primo trattamento e per cui ad oggi non sono disponibili approcci terapeutici con intento curativo, la somministrazione di digossina, a dosi ben tollerate, riduce la dimensione dei cluster di CTC nel sangue, attraverso la ridotta espressione di geni coinvolti nell'adesione cellula-cellula, quindi possibilmente influenzando la colonizzazione metastatica e la prognosi del paziente.

Dal punto di vista clinico, nonostante l'effetto significativo, ma lieve, della digossina sulla dimensione dei cluster di CTC e la mancanza di una verifica diretta degli effetti a livello clinico nelle pazienti (sebbene abbiano dimostrato una ridotta metastatizzazione in seguito alla parziale dissoluzione dei cluster di CTC indotta dalla digossina in modelli preclinici in vivo), questi risultati suggeriscono che in futuro lo studio approfondito di approcci volti ad interferire con il potenziale metastatico delle CTC, attraverso l'interferenza con la dimensione dei cluster di CTC, potrebbe rappresentare un valido approccio per prevenire la colonizzazione metastatica. La letteratura scientifica che indica una correlazione tra la presenza di CTC e la prognosi dei pazienti suggerisce infatti che la digossina potrebbe influenzare la prognosi delle pazienti. Tuttavia, altri fattori potrebbero dover essere tenuti in considerazione, come l'effetto dell'interferenza con l'adesione cellula-cellula sul tumore primario. In ogni caso, dal punto di vista biologico, questo studio fornisce una prova del fatto che è possibile dissolvere farmacologicamente un aggregato di CTC attraverso l'inibizione della Na/K ATPasi, così da ridurre il potenziale metastatico di queste cellule tumorali grazie al riposizionamento di un farmaco utilizzato in ambito cardiologico. Gli autori suggeriscono inoltre l'utilità di studi futuri volti a testare farmaci più potenti o più selettivi, che potrebbero avere effetti più evidenti, dosaggi più elevati o trattamenti prolungati, al fine di disegnare approcci nuovi, efficaci e ottimizzati.

Referenza. Digoxin for reduction of circulating tumor cell cluster size in metastatic breast cancer: a proof-of-concept trial. *Christian Kurzeder, Bich Doan Nguyen-Sträuli, Ilona Krol, Alexander Ring, Francesc Castro-Giner, Manuel Nüesch, Simran Asawa, Yu Wei Zhang, Selina Budinjas, Ana Gvozdenovic, Maren Vogel, Angela Kohler, Cvetka Grašič Kuhar, Fabienne D. Schwab, Viola Heinzelmann-Schwarz, Walter Paul Weber, Christoph Rochlitz, Denise Vorburger, Heike Frauchiger-Heuer, Isabell Witzel, Andreas Wicki, Gabriela M. Kuster, Marcus Vetter, Nicola Aceto*. Nature medicine 2025. doi: 10.1038/s41591-024-03486-6.

What's new in science?

Vaccini personalizzati contro il tumore renale: risultati di un clinical trial di fase I.

Uno studio clinico di fase I ha valutato sicurezza, effetto sull'attività del sistema immunitario ed efficacia di un vaccino tumorale personalizzato in pazienti con tumore renale sottoposti ad intervento chirurgico per rimuovere il tumore primario e ad alto rischio di recidiva. I risultati hanno mostrato che il vaccino era nel complesso ben tollerato, efficace nel controllare la recidiva fino a tre anni dopo l'intervento chirurgico e in grado di stimolare una risposta immunitaria antitumorale.

Quando le mutazioni in una cellula tumorale portano alla generazione di proteine anomale, non presenti nelle cellule normali, queste possono essere riconosciute dal sistema immunitario. Queste proteine sono chiamate neoantigeni e possono stimolare una risposta immunitaria specifica contro la cellula tumorale che esprime quella proteina/neoantigene. I neoantigeni possono essere sfruttati per produrre vaccini tumorali personalizzati, in grado di stimolare una risposta immunitaria specifica, guidata dal neoantigene, e quindi più efficace e meno tossica.

Scoperta principale. In un articolo recente, gli autori riportano i risultati di uno studio clinico di fase I volto a valutare sicurezza, effetto sull'attività del sistema immunitario ed efficacia antitumorale di un vaccino tumorale personalizzato, contro dei neoantigeni, in pazienti con tumore renale sottoposti a resezione chirurgica del tumore primario e ad alto rischio di recidiva. I risultati hanno mostrato che il vaccino era nel complesso ben tollerato, efficace nel controllare la recidiva fino a tre anni dopo l'intervento chirurgico e in grado di stimolare una risposta immunitaria antitumorale.

Dettagli. Nove pazienti con tumore renale in stadio avanzato sono stati arruolati nello studio. I campioni del tumore primario (e, nei due pazienti che avevano metastasi, anche i campioni metastatici) sono stati sequenziati per identificare le mutazioni, che sono state quindi analizzate per valutare la probabilità che generassero neoantigeni. Da queste sequenze mutate, sono stati generati dei frammenti di proteina, raggruppati (in quattro diversi gruppi; ogni gruppo di peptidi somministrato al paziente ne conteneva cinque diversi) e somministrati ai pazienti, da soli o in combinazione con un immunoterapico (ipilimumab).

Sicurezza ed efficacia. Non sono stati osservati eventi avversi severi (si sono manifestate reazioni infiammatorie cutanee a livello del sito di iniezione e sintomi simil-influenzali transitori) e nessuno dei nove pazienti ha manifestato recidiva (follow-up mediano di 40.2 mesi dopo chirurgia). Non si sono osservate differenze tra i pazienti vaccinati (per via intradermica o sottocutanea) con il vaccino soltanto e quelli che hanno ricevuto anche ipilimumab.

Reattività del sistema immunitario. Come reagiva il sistema immunitario? I linfociti T sono stati isolati dai pazienti a tempi diversi dopo la vaccinazione ed è stata analizzata la loro reattività, ex vivo, sia contro i peptidi del pool che contro le cellule tumorali del paziente. Sebbene i tempi di reazione variassero, i linfociti T reagivano in seguito alla stimolazione. Ancora una volta, non si osservava alcuna differenza nei pazienti che ricevevano solo il vaccino rispetto a quelli che ricevevano anche ipilimumab. Tra i neoantigeni valutati, le mutazioni driver più comunemente riscontrate in questo tipo di tumore mostravano un'elevata immunogenicità (elevata capacità di indurre una risposta immunitaria) quando testate in vitro. Analisi comparative, prima e dopo la vaccinazione, della reazione infiammatoria cutanea a livello del sito di iniezione hanno evidenziato una significativa infiltrazione da parte delle cellule immunitarie (mieloidi e linfoidi).

Inoltre, le analisi del sangue dei pazienti vaccinati hanno rivelato un aumento della concentrazione di citochine coinvolte nell'attività dei linfociti T e nella citotossicità. E' importante sottolineare che in seguito alla vaccinazione aumentava anche la concentrazione dei fattori coinvolti nel sopprimere la risposta immunitaria, indicando

una modificazione coordinata della risposta immunitaria dopo la vaccinazione. Infine, la risposta del sistema immunitario alla vaccinazione era duratura: fino a tre anni dopo la vaccinazione, i linfociti T espansi in seguito alla vaccinazione erano in grado di riconoscere, in vitro, i tumori dei pazienti (nel complesso, la reattività al tumore era rilevata nel 77.8% dei pazienti).

Conclusioni. Sebbene il numero di pazienti coinvolti sia limitato, i risultati di questo studio sono promettenti. Gli autori hanno mostrato l'efficacia clinica di un approccio basato sul sequenziamento del tessuto tumorale dei pazienti per la generazione di vaccini personalizzati, a base di peptidi, in grado di stimolare in maniera specifica il sistema immunitario stesso dei pazienti contro il tumore e prevenire la recidiva in pazienti ad alto rischio. E' interessante notare che lo studio è stato condotto in pazienti con un tipo di tumore -il tumore renale- noto per essere caratterizzato da un basso numero di mutazioni, quindi un tumore con una minore probabilità di produrre neoantigeni per stimolare il sistema immunitario. Questi risultati suggeriscono il potenziale di questo approccio in un setting adiuvante, ad esempio in presenza di micrometastasi, dopo l'intervento chirurgico, per eliminare una potenziale malattia residua, clinicamente non rilevabile, magari in combinazione con immunoterapia (diversa da ipilimumab, che ha mostrato scarsa efficacia) per potenziare la risposta immunitaria.

"questi risultati suggeriscono il potenziale di questo approccio nel setting adiuvante, ad esempio in presenza di micrometastasi, dopo la chirurgia, per eliminare l'eventuale malattia residua non rilevabile clinicamente"

Referenza. A neoantigen vaccine generates antitumour immunity in renal cell carcinoma. *David A Braun, Giorgia Moranzoni, Vipheaviny Chea, Bradley A McGregor, Eryn Blass, Chloe R Tu, Allison P Vanasse, Cleo Forman, Juliet Forman, Alexander B Afeyan, Nicholas R Schindler, Yiwen Liu, Shuqiang Li, Jackson Southard, Steven L Chang, Michelle S Hirsch, Nicole R LeBoeuf, Oriol Olive, Ambica Mehndiratta, Haley Greenslade, Keerthi Shetty, Susan Klaeger, Siranush Sarkizova, Christina B Pedersen, Matthew Mossanen, Isabel Carulli, Anna Tarren, Joseph Duke-Cohan, Alexis A Howard, J Bryan Iorgulescu, Bohoon Shim, Jeremy M Simon, Sabina Signoretti, Jon C Aster, Liudmila Elagina, Steven A Carr, Ignaty Leshchiner, Gad Getz, Stacey Gabriel, Nir Hacohen, Lars R Olsen, Giacomo Oliveira, Donna S Neuberg, Kenneth J Livak, Sachet A Shukla, Edward F Fritsch, Catherine J Wu, Derin B Keskin, Patrick A Ott, Toni K Choueiri*. Nature 2025. doi: 10.1038/s41586-024-08507-5.

What's new from IEO Researchers?

Le proteine TBC1D tra metabolismo cellulare e aggressività della cellula tumorale.

Le cellule tumorali sono dotate di una "plasticità metabolica", ovvero la capacità di adattarsi alle elevate richieste energetiche che correlano con la tumorigenicità, riprogrammando il loro metabolismo. Le proteine TBC1D giocano un ruolo fondamentale nel regolare il passaggio di molecole attraverso la membrana cellulare e, attraverso questa regolazione, influiscono sullo stato metabolico della cellula.
In un articolo recente di Lupi, Avanzato, Confalonieri et al., gli autori, co-diretti da Pier Paolo di Fiore -Group Leader al dipartimento di oncologia sperimentale di IEO e professore all'Università di Milano- e Letizia Lanzetti (Candiolo Cancer institute), hanno scoperto che le proteine TBC1D -e in particolare TBC1D7- sono coinvolte nella profonda modificazione del metabolismo cellulare che correla con la prognosi delle pazienti con cancro al seno triplo-negativo (TNBC): ovvero, un elevato livello di espressione della proteina TBC1D7 nella cellula tumorale modifica il trasporto intracellulare della proteina GLUT1, alterando quindi la sua abbondanza a livello della membrana cellulare e sostenendo i cambiamenti del metabolismo della cellula tumorale (verso il pathway glicolitico) che caratterizzano una malattia aggressiva.

Pier Paolo Di Fiore e Letizia Lanzetti

Sebbene il TNBC sia nel complesso un tumore aggressivo, dal punto di vista clinico è piuttosto eterogeneo ed alcune pazienti hanno una prognosi migliore. In questo scenario, innanzi tutto, TBC1D7 rappresenta un candidato promettente per la gestione clinica delle pazienti, identificando le pazienti con una prognosi migliore che potrebbero evitare trattamenti inutilmente aggressivi. In secondo luogo, la correlazione tra TBC1D7 e il metabolismo cellulare rende questa proteina un interessante potenziale target farmacologico per interferire con le alterazioni del metabolismo cellulare che caratterizzano le cellule tumorali.

TELL ME MORE!

Sfruttando il dataset METABRIC, gli autori hanno osservato che l'espressione delle proteine TBC1D (proteine contenenti il dominio TBC, TBC1D) era spesso alterata nel cancro al seno triplo-negativo (TNBC) rispetto agli altri sottotipi molecolari e rifletteva la prognosi delle pazienti; in particolare, livelli elevati di TBC1D correlavano con una prognosi peggiore.
Inoltre, l'espressione di (undici) geni TBC1D era associata con un profilo metabolico alterato delle cellule nel tumore al seno. Un gruppo di geni TBC1D correlava con livelli elevati di metaboliti del pathway glicolitico e con geni tipicamente sovraespressi nel TNBC, mentre un altro gruppo era associato con livelli elevati di metaboliti del pathway di ossidazione degli acidi grassi e geni generalmente poco espressi nel TNBC. Analisi ulteriori hanno rivelato una relazione causale tra l'espressione delle proteine TBC1D e il pathway glicolitico: il silenziamento dei geni TBC1D induceva una down-regolazione del pathway glicolitico e riduceva l'attività mitocondriale, indicando che in assenza di TBC1D la cellula

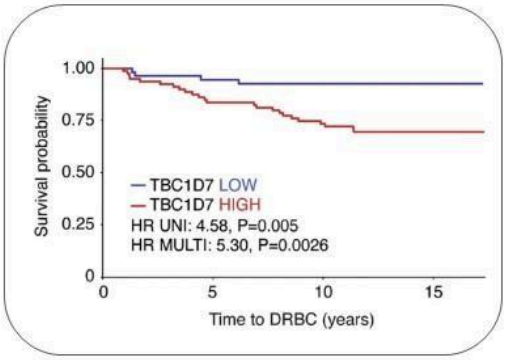


Figure adapted from Lupi et al., an open access article under the CC-BY licence

tumorale riprogrammava il proprio metabolismo verso uno stato meno energetico.
Focalizzandosi in maniera specifica su TBC1D7, gli autori hanno mostrato che la modulazione del metabolismo cellulare da parte di TBC1D7 avveniva, in parte, attraverso la regolazione del trafficking di membrana e la risultante variazione dell'abbondanza di GLUT1 sulla superficie cellulare e, in parte, attraverso una regolazione a livello trascrizionale, sebbene non sia chiaro in che modo TBC1D7 regoli trascrizionalmente il reprogramming cellulare. Infatti, sfruttando un mutante di TBC1D7 incapace di legare il complesso proteico (TSC1/TSC2) che regola l'attività di mTORC1, gli autori hanno mostrato che non vi era alcuna correlazione con mTORC1, il cui pathway è invece noto essere represso da TBC1D7.
Inoltre, utilizzando un'ampia coorte di campioni di pazienti IEO, gli autori hanno confermato che, a livello proteico così come a livello dell'RNA, l'espressione di TBC1D7 distingueva le pazienti con TNBC in due gruppi a seconda della prognosi. Inoltre, il silenziamento di TBC1D7 influenzava il pathway glicolitico solo di quelle cellule che (prima del silenziamento) avevano alti livelli di TBC1D7, mentre non aveva praticamente alcun effetto in quelle cellule che (prima del silenziamento) avevano livelli bassi di TBC1D7, indicando che in queste cellule sono necessari livelli di espressione elevati di TBC1D7 per mantenere la glicolisi. In linea con questi dati, i geni del metabolismo cellulare -in particolare, molti geni del pathway glicolitico- erano down-regolati nelle cellule silenziate per TBC1D7.

Referenza. TBC1 domain-containing proteins are frequently involved in triple-negative breast cancers in connection with the induction of a glycolytic phenotype. *Mariadomenica Lupi #, Daniele Avanzato #, Stefano Confalonieri #, Flavia Martino, Rosa Pennisi, Emanuela Pupo, Valentina Audrito, Stefano Freddi, Giovanni Bertalot, Francesca Montani, Bronislava Matoskova, Sara Sigismund, Pier Paolo Di Fiore*, Letizia Lanzetti*.* Cell Death Dis 2024. doi: 10.1038/s41419-024-07037-2.

Da studi preclinici, un nuovo biomarcatore e target farmacologico nel tumore della vescica.

Pece Lab

Sebbene la maggioranza (75%) dei tumori della vescica sia non invasiva ed abbia nel complesso una buona prognosi, questi tumori spesso evolvono in una malattia invasiva, con una cattiva prognosi , nonostante le opzioni terapeutiche attualmente disponibili, che sono accompagnate da severi effetti collaterali e scarsa qualità di vita. Una delle difficoltà nel trattamento ottimale dei pazienti con tumore della vescica è legata alla mancanza di biomarcatori, che impedisce di stimare il rischio che la malattia non invasiva diventi invasiva e quindi di distinguere i pazienti che potrebbero semplicemente sottoporsi a sorveglianza attiva da coloro che sono ad alto rischio e necessitano di un trattamento più aggressivo. Un altro aspetto critico è la scarsità di terapie mirate ed efficaci.

In uno studio recente di Tucci, Pennisi et al., finanziato da AIRC, gli autori, co-diretti da Salvatore Pece e Daniela Tosoni –rispettivamente PI e Ricercatrice al dipartimento di oncologia sperimentale di IEO–, hanno scoperto che la proteina NUMB è un marcatore prognostico e predittivo di progressione nel tumore della vescica, dato che la sua assenza è associata con un elevato rischio di progressione della malattia da non invasiva ad invasiva. Inoltre, NUMB gioca un ruolo attivo nella tumorigenesi, dato che la sua assenza aumenta l'aggressività delle cellule tumorali già trasformate ed è sufficiente da sola ad indurre il tumore.

Hanno definito i meccanismi molecolari implicati e propongono NUMB –e il pathway associato RhoA-Rock-YAP– come nuovo target terapeutico per contrastare la progressione del tumore.
Identificando NUMB come nuovo biomarcatore di progressione/aggressività della malattia, questo studio contribuisce a migliorare l'attuale gestione clinica dei pazienti con tumore vescicale. La presenza/assenza di NUMB –così come la firma molecolare di 27 geni associata alla perdita di NUMB– potrebbe infatti essere sfruttata in un contesto clinico, insieme agli attuali parametri clinico-patologici disponibili, come marcatore per distinguere i pazienti ad alto rischio da quelli a basso rischio. Inoltre, per via del suo ruolo attivo nel processo di tumorigenesi, NUMB rappresenta non solo un biomarcatore, ma anche un potenziale nuovo target farmacologico per arrestare la progressione del tumore vescicale. A questo riguardo, è interessante notare che diversi farmaci che interferiscono con i pathway associati alla perdita di NUMB sono già utilizzati in ambito clinico, come, ad esempio, Verteporfin (inibitore di YAP), impiegato nel trattamento di condizioni oftalmologiche, o fasudil (inibitore di ROCK), in sperimentazione clinica per malattie vascolari e neurodegenerative, indicando la possibilità di un rapido riposizionamento nel trattamento del tumore della vescica.

"La nostra scoperta – commenta Salvatore Pece, PI di IEO e professore ordinario all'università di Milano– ha un forte e immediato potenziale di applicazione nella pratica clinica, dimostrando che i tumori vescicali superficiali e quelli profondi rappresentano stadi differenti di un unico processo patologico che evolve nel tempo, guidato fin dal principio da specifici meccanismi molecolari che possono essere ostacolati con farmaci mirati. Diventa quindi fondamentale identificare i meccanismi biologici alla base di questa evoluzione e sviluppare nuovi marcatori molecolari per identificare i pazienti con caratteristiche specifiche di aggressività".
"Noi abbiamo dimostrato che è possibile inibire la capacità proliferativa e invasiva delle cellule tumorali prive di NUMB – aggiunge Daniela Tosoni, ricercatrice di IEO e dell'università di Milano– utilizzando farmaci in grado di colpire questo complesso circuito molecolare a diversi livelli. I tumori della vescica privi di NUMB sono sì molto aggressivi, ma anche molto vulnerabili".
"Abbiamo anche identificato – continua Salvatore Pece – una nuova firma molecolare che consentirà di identificare con accurata precisione i pazienti che potranno beneficiare di trattamenti mirati con nuovi farmaci che colpiscono in maniera specifica i meccanismi molecolari che sono attivati in seguito alla perdita di NUMB".
"Lo studio, che ha visto impegnati in uno sforzo comune scienziati e clinici del nostro istituto – conclude il professor Roberto Orecchia, Direttore dello IEO di Milano – è un risultato straordinario. Abbiamo già brevettato la nuova firma molecolare emersa da queste ricerche e stiamo per avviare studi clinici per validarne l'utilizzo come marcatore, per identificare i pazienti ad alto rischio di progressione di malattia che potranno beneficiare nel prossimo futuro di una nuova prospettiva terapeutica con farmaci più precisi e mirati".
Leggi qui il comunicato (solo in italiano).

TELL ME MORE!

Ricercatori IEO hanno precedentemente dimostrato un ruolo della proteina NUMB nella tumorigenesi del cancro al seno (Filippone et al., J Cell Biol 2022). In questo lavoro, gli autori hanno osservato che l'espressione di NUMB era spesso più bassa in campioni di tumore della vescica rispetto ai tessuti sani e i livelli di espressione correlavano con l'aggressività della malattia e la mortalità: una bassa espressione di NUMB in tumori non invasivi prediceva la progressione in malattia aggressiva, invasiva, indipendentemente da altri fattori (come sesso, età, stadio TNM), mostrando la sua utilità nella stratificazione dei pazienti in classi di rischio.
La caratterizzazione trascrizionale di linee cellulari, sia maschili che femminili, di tumore della vescica NUMB-low e NUMB-high ha permesso l'identificazione di una firma molecolare di 27 geni in grado di predire un rischio elevato di progressione della malattia, distinguendo pazienti NUMB-low, con maggiore rischio di progressione in malattia invasiva, da pazienti NUMB-high.
Analisi dettagliate dei meccanismi molecolari coinvolti hanno rivelato i pathway cellulari

implicati: la ridotta espressione di NUMB determinava l'iper-attivazione del pathway RHOA/ROCK, che a sua volta influenzava il citoscheletro di actina. Ciò inibiva il pathway Hippo, con conseguente attivazione del pathway di YAP e del programma di transizione epitelio-mesenchimale (EMT), verosimilmente responsabile dell'acquisizione di un comportamento cellulare più aggressivo/invasivo nelle cellule tumorali NUMB-low. L'inibizione farmacologica mirata, a diversi stadi, della cascata molecolare a valle della perdita di NUMB limitava in maniera significativa l'invasività delle cellule tumorali; questi farmaci colpivano in maniera selettiva le cellule tumorali prive di NUMB, essendo al tempo stesso inefficaci sulle cellule che esprimevano NUMB.

Le cellule tumorali prive di NUMB avevano anche una differente morfologia rispetto a quelle che lo esprimevano: erano meno rotondeggianti, irregolari, con protrusioni, una superficie più estesa e perdevano l'adesione cellula-cellula, indicando un fenotipo invasivo. L'inibizione farmacologica dei pathway cellulari associati a NUMB e la ri-espressione ectopica di NUMB invertivano il fenotipo invasivo.

La perdita di NUMB non solo induceva la comparsa di lesioni neoplastiche infiltranti, ma aumentava anche l'aggressività delle cellule già trasformate, accelerando la progressione da tumori non invasivi a tumori invasivi.

Le scoperte nei modelli murini, in vitro e in vivo, sono state confermate in modelli preclinici di origine umana, evidenziandone la rilevanza clinica.

Quindi, impiegando colture cellulari in vitro murine e di origine umana, così come modelli murini preclinici in vivo e dati dei pazienti, inibizione farmacologica e silenziamento genico, questo lavoro mostra che, in linea con studi precedenti che dimostrano il ruolo di NUMB nella tumorigenesi del cancro al seno, NUMB agisce come oncosoppressore nel tumore della vescica; la sua perdita infatti non solo accelera la progressione della malattia da non invasiva ad invasiva, ma è anche sufficiente da sola ad indurre tumorigenesi. NUMB rappresenta al tempo stesso un biomarcatore di aggressività della malattia e un target farmacologico, nel tumore della vescica e, forse, anche in altri tipi di tumore.

Referenza. Loss of NUMB drives aggressive bladder cancer via a RHOA/ROCK/YAP signaling axis. *Tucci FA, Pennisi R, Rigiracciolo DC, Filippone MG, Bonfanti R, Romeo F, Freddi S, Guerrera E, Soriani C, Rodighiero S, Gunby RH, Jodice G, Sanguedolce F, Renne G, Fusco N, Di Fiore PP, Pruneri G, Bertalot G, Musi G, Vago G, Tosoni D, Pece S*. Nat Commun. 2024. doi: 10.1038/s41467-024-54246-6.

In che modo le cellule gestiscono l'aneuploidia? La caratterizzazione dei meccanismi molecolari implicati rivela dei punti deboli delle cellule tumorali, da poter sfruttare a scopo terapeutico.

Stefano Santaguida e Marica Ippolito.

Un numero alterato di cromosomi –aneuploidia– è una caratteristica comune nelle cellule tumorali, induce instabilità genomica, danno al DNA e stress cellulare. Per poter sopravvivere e proliferare, le cellule aneuploidi devono trovare il modo di gestire queste anomalie. Comprendere a livello molecolare i meccanismi attraverso cui le cellule gestiscono lo stress associato all'aneuploidia potrebbe rivelare dei punti deboli delle cellule aneuploidi tumorali potenzialmente sfruttabili a scopo terapeutico per eliminare in maniera selettiva le cellule tumorali aneuploidi.

In due articoli recenti, di Ippolito, Zerbib, et al. pubblicati in *Cancer Discovery* e *Nature Communications*, gli autori co-supervisionati da Stefano Santaguida –Group Leader al Dipartimento di Oncologia Sperimentale di IEO e professore all'Università di Milano– e Uri Ben-David –dell'università di Tel Aviv– hanno effettuato una caratterizzazione genomica, trascrittomica e proteomica delle cellule aneuploidi, rivelando che le cellule aneuploidi –sia non trasformate che maligne– hanno evoluto specifici meccanismi per gestire lo stress associato con l'aneuploidia.

In particolare, gli autori hanno dimostrato che, da un lato, le cellule aneuploidi attivano meccanismi per degradare l'eccesso di RNA e proteine sintetizzati, che includono l'attivazione del proteasoma; dall'altro,

aumentando l'attività del pathway RAF/MEK/ERK, le cellule aneuploidi riescono ad ovviare all'elevato danno al DNA che caratterizza la condizione aneuploide, o che si verifica ad esempio durante il trattamento chemioterapico causando chemioresistenza.
L'identificazione di questi meccanismi di sopravvivenza delle cellule aneuploidi ha implicazioni terapeutiche rilevanti. Infatti, da un lato, l'aneuploidia rende queste cellule sensibili agli inibitori del pathway RAF/MEK/ERK e l'inibizione farmacologica di RAF/MEK/ERK rende a sua volta le cellule aneuploidi sensibili a farmaci che inducono danno al DNA –come i chemioterapici– suggerendo la possibilità di nuove possibili combinazioni terapeutiche. Dall'altro, la necessità di attivare i meccanismi identificati per compensare l'eccesso di RNA e proteine associato con un cromosoma di troppo e poter sopravvivere rende le cellule aneuploidi vulnerabili e l'inibizione farmacologica dei meccanismi di degradazione di RNA e proteine in eccesso uccide le cellule aneuploidi. Questi punti deboli delle cellule aneuploidi possono essere sfruttati in un contesto clinico; ad esempio, alcuni inibitori del proteasoma –come bortezomib– sono clinicamente approvati ed utilizzati per il trattamento dei pazienti. Inoltre, i risultati indicano che l'aneuploidia (in particolare, il grado di aneuploidia) potrebbe rappresentare un marcatore per identificare i pazienti che risponderanno a terapie con inibitori del proteasoma.

TELL ME MORE!

Prima di tutto, gli autori hanno sviluppato un sistema adeguato per analizzare l'aneuploidia in cellule non trasformate, fornendo allo stesso tempo alla comunità scientifica uno strumento utile per lo studio dell'aneuploidia. Attraverso un trattamento chimico, hanno indotto, in maniera casuale, aneuploidia in cellule RPE; successivamente, hanno selezionato sei popolazioni cellulari con diversi gradi di aneuploidia: cellule con bassi livelli di aneuploidia (LLA) e cellule con elevati livelli di aneuploidia (HLA, con cariotipo complesso).
La caratterizzazione molecolare (genomica, trascrittomica e proteomica) di queste cellule ha permesso di identificare una signature trascrittomica associata allo stato aneuploide in generale piuttosto che agli specifici cromosomi persi o acquisiti. Questa signature di aneuploidia era caratterizzata dall'up-regolazione dei geni di risposta e di riparo del danno al DNA (DDR), up-regolazione dei pathway del metabolismo dell'RNA e dello stress proteotossico (così come la down-regolazione di geni associati alla proliferazione cellulare), suggerendo che le cellule aneuploidi fossero in grado di gestire livelli elevati di danno al DNA (DD). La caratterizzazione di queste cellule in termini di sensibilità ai farmaci ha rivelato che le cellule HLA mostravano un maggior livello di DD, ma grazie all'up-regolazione dei geni di DDR avevano anche una maggiore capacità di tollerarlo ed erano quindi più resistenti a farmaci che inducono DD, come topotecan e etoposide –due chemioterapici comunemente usati in ambito clinico– e olaparib –un inibitore di PARP clinicamente approvato. Questi risultati sono stati confermati in altri modelli di cellule aneuploidi, sia non trasformate che tumorali. Nonostante la maggiore resistenza delle cellule aneuploidi ai farmaci che inducevano DD, che potevano proliferare e sopravvivere grazie all'up-regolazione dei geni di DDR, queste cellule mostravano altri punti deboli, come la maggiore sensibilità a inibitori del pathway RAF/MEK/ERK (particolarmente evidente in cellule HLA) e, nello specifico, ad inibizione di c-RAF. c-RAF era attivato in cellule HLA ma non in cellule LLA. L'attivazione di c-RAF era un evento precoce dopo l'induzione dell'aneuploidia, suggerendo che fosse un meccanismo adattativo, sia in cellule aneuploidi non trasformate che tumorali, necessaria per superare il DD. Generalmente c-RAF è attivato dalla DDR e, infatti, l'inibitore di c-RAF in combinazione con farmaci che inducono DD uccideva le cellule aneuploidi: l'inibizione di c-RAF rendeva le cellule aneuploidi sensibili ai farmaci che inducono DD come etoposide e olaparib. Le cellule aneuploidi -sia quelle non trasformate che quelle tumorali- erano anche sensibili a inibitori di MEK clinicamente approvati come trametinib (e selumetinib) e all'inibitore di ERK ulixertinib. L'iperattivazione del pathway MEK/ERK rappresentava quindi il meccanismo di resistenza delle cellule aneuploidi e l'inibizione di questo pathway sensibilizzava le cellule ai farmaci che inducono DD -come la chemioterapia-, supportando l'efficacia antitumorale della combinazione di inibitori di DDR e del pathway MEK/ERK. Infine, analizzando i dataset di espressione genica dei pazienti, hanno osservato che la resistenza a olaparib corrispondeva all'iperattivazione del pathway MEK/ERK, in

maniera specifica nelle cellule tumorali aneuploidi. (Zerbib, Ippolito et al., Nature communications 2024).

La caratterizzazione molecolare delle cellule aneuploidi ha inoltre evidenziato l'up-regolazione di geni associati con la modulazione di RNA e proteine, sia in cellule aneuploidi non trasformate che in cellule aneuploidi tumorali; nello specifico, geni associati al metabolismo dell'RNA e al silenziamento genico, così come geni del pathway UPR (*unfolded protein response*) e della degradazione delle proteine, indicando che le cellule aneuploidi sfruttano questi meccanismi per attenuare l'eccesso di RNA e proteine associato con l'aneuploidia. Inoltre, in accordo con studi precedenti (Senger et al., Elife 2022), il meccanismo di compensazione era particolarmente importante per le proteine che facevano parte di complessi multiproteici. In particolare, per quanto riguarda i livelli di RNA, le cellule aneuploidi non solo mostravano livelli più elevati di sintesi dell'RNA, ma anche una maggiore degradazione dell'RNA dovuta alla sovraespressione del pathway NMD (*nonsense-mediated decay*), indotto dalla DDR (*DNA damage response*) attivata in conseguenza dell'aneuploidia. La degradazione dell'RNA mediata da NMD consentiva alle cellule tumorali aneuploidi di proliferare, per cui le cellule aneuploidi erano maggiormente dipendenti da questo pathway e quindi più sensibili ai farmaci che lo inibivano. Anche nei tumori umani l'aneuploidia era associata con la sovraespressione dei geni NMD. Oltre alla degradazione dell'RNA mediata da NMD, anche i meccanismi di silenziamento dell'espressione genica via miRNA giocavano un ruolo chiave nel compensare l'RNA in eccesso. Le cellule aneuploidi affrontavano quindi lo stress associato con l'eccesso di RNA attivando meccanismi di degradazione dell'RNA mediati da NMD e miRNA, che se da un lato permettevano alle cellule aneuploidi di sopravvivere, dall'altro rappresentavano il loro punto debole, con cui poter interferire per eliminarle. Analogamente, l'eccesso di proteine associato all'aneuploidia induceva uno stress proteotossico, a cui le cellule reagivano attivando il pathway UPR -la risposta primaria delle cellule aneuploidi allo stress proteotossico- che determinava l'attenuazione della sintesi delle proteine. Inoltre, queste cellule mostravano una maggiore attivazione della degradazione delle proteine mediata dal proteasoma, una maggiore dipendenza delle cellule aneuploidi dall'attività del proteasoma e, di conseguenza, una maggiore sensibilità delle cellule aneuploidi all'inibizione del proteasoma. Le cellule aneuploidi up-regolavano l'attività del proteasoma in risposta allo stress proteotossico e questo le rendeva più sensibili all'inibizione del proteasoma: bortezomib -inibitore del proteasoma clinicamente approvato- uccideva le cellule aneuploidi Infine, la maggioranza dei pazienti in cui il trattamento con bortezomib induceva una risposta completa avevano tumori con un grado di aneuploidia più elevato rispetto a coloro in cui la malattia progrediva, evidenziando che la risposta all'inibitore del proteasoma bortezomib correlava con il grado di aneuploidia, indicando che quest'ultimo potrebbe essere usato come marcatore per predire la risposta alla terapia con inibitori del proteasoma, sottolineando ulteriormente la rilevanza clinica di queste scoperte (Ippolito, Zerbib et al., Cancer Discovery 2024).

Referenze.

1) Increased RNA and Protein Degradation Is Required for Counteracting Transcriptional Burden and Proteotoxic Stress in Human Aneuploid Cells. *Marica Rosaria Ippolito #, Johanna Zerbib #, Yonatan Eliezer, Eli Reuveni, Sonia Viganò, Giuseppina De Feudis, Eldad D Shulman, Anouk Savir Kadmon, Rachel Slutsky, Tiangen Chang, Emma M Campagnolo, Silvia Taglietti, Simone Scorzoni, Sara Gianotti, Sara Martin, Julia Muenzner, Michael Mülleder, Nir Rozenblum, Carmela Rubolino, Tal Ben-Yishay, Kathrin Laue, Yael Cohen-Sharir, Ilaria Vigorito, Francesco Nicassio, Eytan Ruppin, Markus Ralser, Francisca Vazquez, Stefano Santaguida*, Uri Ben-David**. Cancer Discov 2024. doi: 10.1158/2159-8290.CD-23-0309. **2)** Human aneuploid cells depend on the RAF/MEK/ERK pathway for overcoming increased DNA damage. Johanna Zerbib #, Marica Rosaria Ippolito #, Yonatan Eliezer, Giuseppina De Feudis, Eli Reuveni, Anouk Savir Kadmon, Sara Martin, Sonia Viganò, Gil Leor, James Berstler, Julia Muenzner, Michael Mülleder, Emma M Campagnolo, Eldad D Shulman, Tiangen Chang, Carmela Rubolino, Kathrin Laue, Yael Cohen-Sharir, Simone Scorzoni, Silvia Taglietti, Alice Ratti, Chani Stossel, Talia Golan, Francesco Nicassio, Eytan Ruppin, Markus Ralser, Francisca Vazquez, Uri Ben-David*, Stefano Santaguida*. Nat Commun 2024. doi: 10.1038/s41467-024-52176-x.

What's new from IEO Researchers?

Terapia molecolare per il tumore al seno HER2-negativo: risultati di un clinical trial di fase III.

Il tumore al seno HER2 (human epidermal growth factor 2)-negativo è il sottotipo di tumore al seno più comune. Questi tumori sono piuttosto eterogenei in termini di livelli di espressione di HER2 e, sulla base dell'espressione di HER2 (misurata tramite immunoistochimica e in situ hybridization), possono essere classificati in HER2-low e HER2-ultralow. L'attuale terapia standard per le pazienti con tumore al seno HER2-negativo metastatico prevede una terapia endocrina più inibitori di CDK4/6. Lo schema di trattamento previsto in caso di progressione della malattia non è però ben definito.

In un articolo recentemente pubblicato sulla rivista *The New England Journal of Medicine*, nell'ambito di una collaborazione internazionale coordinata da Giuseppe Curigliano -Vicedirettore Scientifico IEO e Direttore della Divisione Sviluppo Nuovi Farmaci per Terapie Innovative di IEO-, nel contesto dello studio clinico di fase III DESTINY-Breast06, gli autori hanno dimostrato l'efficacia e la sicurezza di trastuzumab deruxtecan (TDX), un anticorpo farmaco-coniugato diretto contro la proteina HER2, rispetto alla chemioterapia, in pazienti con tumore al seno positivo per il recettore degli ormoni, metastatico con livelli molto bassi di proteina HER2.

Giuseppe Curigliano.

TDX ha mostrato benefici significativi sia in pazienti con tumore al seno HER2-low che in pazienti con tumore HER2-ultralow, con una sopravvivenza in assenza di malattia più lunga rispetto alle pazienti trattate con chemioterapia. TDX si è dimostrata più efficace indipendentemente dai livelli di espressione di HER2 (dato che era efficace in tutte le pazienti, sia che fossero HER2-low che HER2-ultralow, probabilmente per via della presenza di HER2 a livello delle membrana cellulare, sebbene a livelli molto bassi), o un precedente trattamento con inibitori di CDK4/6, o lo specifico chemioterapico rispetto a cui è stato effettuato il confronto.

Sulla base dei risultati del precedente studio clinico DESTINY-Breast04, TDX era stato già approvato per il trattamento di pazienti con metastasi in cui la malattia era progredita nonostante la chemioterapia. Nel contesto di questo studio DESTINY-Breast06, gli autori hanno dimostrato l'efficacia di TDX *anche* in pazienti in stadi più precoci del percorso terapeutico, ovvero quelle pazienti che non hanno ancora ricevuto chemioterapia.

TELL ME MORE!

DESTINY-Breast06 è uno studio multicentrico di fase III, che ha reclutato 866 pazienti (713 HER2-low e 153 HER2-ultralow), in 32 centri, con malattia metastatica che è progredita con il trattamento standard. Le pazienti arruolate sono state casualmente assegnate a ricevere TDX (436 pazienti) o chemioterapia (430 pazienti). Gli autori hanno valutato sicurezza ed efficacia del trattamento innanzi tutto in termini di sopravvivenza in assenza di malattia (progression-free survival, PFS) e in secondo luogo in termini di sopravvivenza generale (overall survival, OS). Sono state valutate anche la risposta obiettiva e la durata della risposta. Le analisi sono state effettuate in tutta la popolazione arruolata, ovvero comprendente pazienti HER2-ultralow (con uno score di staining HER2 uguale a 0) e pazienti HER2-low (ovvero con uno score di staining HER2 pari a 1 o 2) e nel sottogruppo HER2-low separatamente.

Efficacia. Il trattamento con TDX era più efficace della chemioterapia, con una PFS significativamente più lunga nelle pazienti trattati con TDX rispetto alle pazienti che ricevevano chemioterapia, sia nel sottogruppo di pazienti con tumore HER2-low che nell'intera popolazione (ovvero comprendente sia HER2-low che HER2-ultralow). I risultati sulla OS raccolti finora non sono maturi; inoltre, è importante considerare che dato che in queste pazienti la sopravvivenza dopo la progressione della malattia è maggiore, eventuali terapie somministrate successivamente avranno molto probabilmente un effetto significativo sulla OS che dovrà essere tenuto in considerazione. I risultati hanno mostrato una maggiore efficacia di TDX anche considerando la durata della risposta e la risposta obiettiva; alcune pazienti trattate con TDX hanno mostrato una risposta completa (mentre nessuna delle pazienti trattate con chemioterapia ha avuto una risposta completa).

Sicurezza. L'incidenza di eventi avversi era simile nelle pazienti trattate con TDX e nelle pazienti trattate con chemioterapia. Tuttavia, era più spesso necessaria una riduzione del dosaggio in caso di trattamento con chemioterapia che con TDX; l'interruzione del trattamento era necessaria più nel gruppo trattato con TDX che nel gruppo di pazienti che ricevevano chemioterapia; eventi avversi seri e fatali correlati al trattamento erano più comuni nelle pazienti trattate con TDX rispetto a quelle sottoposte a chemioterapia.

I risultati di questo studio indicano che nel complesso TDX rappresenta una valida opzione terapeutica per le pazienti con tumore al seno positivo per il recettore degli ormoni, HER2-negativo, in stadi precoci di trattamento, anche se esprimono livelli di HER2 estremamente bassi.

Referenza. Trastuzumab Deruxtecan after Endocrine Therapy in Metastatic Breast Cancer. *A. Bardia, X. Hu, R. Dent, K. Yonemori, C.H. Barrios, J.A. O'Shaughnessy, H. Wildiers, J.-Y. Pierga, Q. Zhang, C. Saura, L. Biganzoli, J. Sohn, S.-A. Im, C. Lévy, W. Jacot, N. Begbie, J. Ke, G. Patel, and G. Curigliano, for the DESTINY-Breast06 Trial Investigators**. The new england journal of medicine 2024. doi: 10.1056/NEJMoa2407086.

What's new from IEO Researchers?

Tucatinib e trastuzumab per il trattamento del tumore al seno in stadio avanzato: risultati di uno studio clinico di fase II.

La sovraespressione della proteina HER2 (*human epidermal growth factor receptor 2*), determinando l'iper-attivazione dei processi cellulari a valle, induce la proliferazione cellulare incontrollata, l'inibizione della morte cellulare e la formazione di metastasi. HER2 costituisce quindi un interessante target farmacologico e, infatti, numerosi studi hanno mostrato l'efficacia clinica di farmaci che interferiscono con l'attività di HER2, nel tumore al seno, nel tumore gastrico e nel tumore del colon-retto.

Dato che circa il 2-5% dei tumori al seno ha mutazioni nella sequenza del gene HER2, soprattutto negli stadi avanzati della malattia, nel contesto di un recente basket trial di fase II (SGNTUC-019, volto a valutare l'efficacia del farmaco contro HER2 tucatinib, in combinazione con trastuzumab, in tumori solidi in stadio avanzato), i ricercatori, tra cui Giuseppe Curigliano –vice-direttore scientifico di IEO, direttore della divisione di Sviluppo Nuovi Farmaci per Terapie Innovative e co-direttore del Programma Nuovi Farmaci– hanno valutato sicurezza ed efficacia del trattamento combinato con Tucatinib (inibitore tirosin-chinasico molto selettivo per HER2) e trastuzumab, in pazienti con tumore al seno metastatico e mutazioni nel gene HER2.

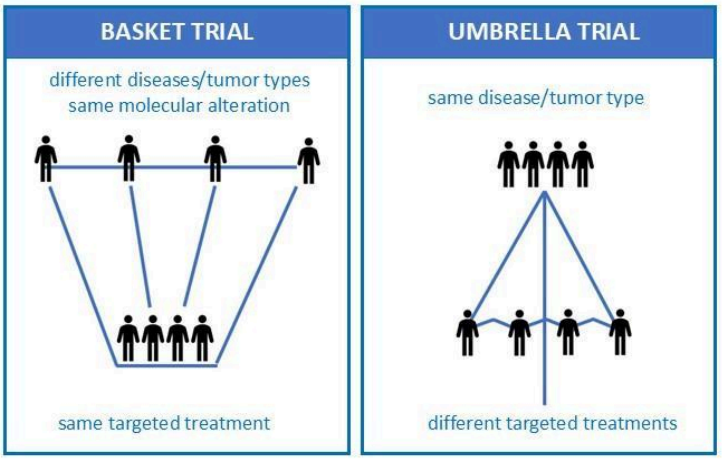
Sebbene siano nel complesso preliminari e necessitino quindi di dati ulteriori in coorti più ampie di pazienti o a follow-up più lunghi, questi risultati propongono il trattamento combinato tucatinib-trastuzumab in pazienti con tumore al seno metastatico e mutazioni del gene HER2 in cui i cicli di terapia precedenti hanno fallito, offrendo così a queste pazienti un'ulteriore opzione terapeutica. Infatti, la combinazione di tucatinib e trastuzumab si è dimostrata efficace nel ridurre la dimensione del tumore e aumentare la sopravvivenza; due pazienti hanno persino mostrato una risposta completa, a sostegno dell'importanza di studi ulteriori.

Tucatinib. Inibitore tirosin-chinasico. Legandosi al recettore HER2, ne inibisce l'attività tirosin chinasica, bloccando così la signaling cellulare a valle. A differenza di altre molecole, tucatinib mostra un'elevata selettività per HER2. Studi preclinici in vitro hanno mostrato che tucatinib uccide solo le cellule che sovraesprimono HER2; in vivo, questo inibitore è in grado di indurre la regressione del tumore in diversi modelli tumorali, sia somministrato da solo che in combinazione con altri farmaci. In particolare, è stata dimostrata una maggiore attività antitumorale se somministrato in combinazione con trastuzumab or docetaxel.

Trastuzumab. Anticorpo specifico diretto contro la proteina HER2. Legandosi in maniera selettiva alla porzione extracellulare di HER2, inibisce la signaling cellulare a valle e, di conseguenza, la proliferazione della cellula tumorale. Trastuzumab ha mostrato efficacia nel rallentare la progressione del tumore quando somministrato da solo e la sua efficacia antitumorale aumenta significativamente quando somministrato in combinazione con chemioterapia.

Basket trial. Diversamente dai tradizionali studi clinici tumore-specifici, in cui i pazienti vengono arruolati e sottoposti a determinati protocolli terapeutici sperimentali sulla base del proprio tipo di tumore, i basket trial sono studi clinici in cui pazienti con la stessa alterazione molecolare sono trattati con lo stesso farmaco molecolare indipendentemente dal tipo di tumore. I basket trial sono un nuovo tipo di clinical trial, evolutosi, in un'ottica di medicina di precisione, con l'avvento e la diffusione delle tecnologie di sequenziamento di ultima generazione, insieme agli "umbrella trial" in cui, invece, diversi tipi di terapie molecolari sono testate (sulla base di specifici biomarcatori) su pazienti con lo stesso tipo di tumore.

Infine, sebbene i risultati della ricerca volta ad identificare biomarcatori di risposta non abbiano permesso di trarre conclusioni definitive, hanno dimostrato la fattibilità del sequenziamento (tramite next-generation sequencing, NGS), sia su campioni di sangue che su sezioni di tessuto tumorale, per l'identificazione di mutazioni oncogeniche nel gene HER2, che potrebbero essere utili per distinguere le pazienti con buone probabilità di rispondere a questo tipo di trattamento.



TELL ME MORE!

Lo studio ha arruolato 31 pazienti con tumore al seno metastatico e mutazioni nel gene HER2 in cui la malattia è progredita nonostante i precedenti cicli di terapia. Le pazienti hanno ricevuto tucatinib e trastuzumab (alcune pazienti –HR-positive– hanno ricevuto anche fulvestrant).

Efficacia. I primi segni di risposta al trattamento sono stati visibili poco dopo l'inizio della terapia; nel 73% delle pazienti arruolate si è osservata una riduzione della dimensione del tumore; il 42% delle pazienti ha mostrato una risposta obiettiva (secondo la valutazione del medico) e due pazienti hanno persino mostrato una risposta completa. Nonostante il periodo di follow-up relativamente breve (durata mediana del follow-up, 15 mesi), la risposta al trattamento appariva duratura: infatti, la valutazione della sopravvivenza in assenza di progressione della malattia (PFS) e della sopravvivenza generale (OS) hanno permesso di stimare una PFS a 12 mesi per il 45% delle pazienti e una OS a 12 mesi per il 74% delle pazienti (nello specifico, la PFS mediana era di 9.5 mesi e la OS mediana era di 21.1 mesi).

Sicurezza. Il trattamento combinato era ben tollerato anche nelle pazienti che erano già state sottoposte ad intensi cicli di terapia. Nel complesso, il 26% delle pazienti manifestava eventi avversi severi correlati al trattamento e nel 10% di queste pazienti gli eventi avversi erano associati al tucatinib. In ogni caso, gli eventi avversi hanno portato all'interruzione della somministrazione di tucatinib solo in due pazienti, sebbene siano state effettuate delle riduzioni del dosaggio. Nessuna delle pazienti arruolate è deceduta per via del trattamento (sebbene undici di loro siano decedute a causa della progressione della malattia).

Analisi di biomarcatori. Una volta valutate sicurezza ed efficacia, i ricercatori hanno effettuato un'analisi esplorativa alla ricerca di potenziali biomarcatori per identificare le pazienti che avevano buone probabilità di rispondere al trattamento (*responder*). Le analisi, tramite NGS, del sangue o del tessuto tumorale hanno permesso l'identificazione di un ampio spettro di mutazioni diverse del gene HER2 (principalmente nel dominio tirosin-chinasico o nel dominio extracellulare) e la contemporanea presenza di altre mutazioni (spesso nei geni CDH1, PIK3CA) nelle *responder*. Sebbene il numero di pazienti sia troppo esiguo per poter trarre delle conclusioni solide riguardo ai biomarcatori, le loro analisi hanno dimostrato che è possibile analizzare tramite NGS campioni tissutali o di sangue al fine di valutare lo stato mutazionale di HER2 e distinguere *responder* e *non-responder*.

Referenza. Tucatinib and trastuzumab in HER2-mutated metastatic breast cancer: a phase 2 basket trial. *Alicia F C Okines, Giuseppe Curigliano, Nobumasa Mizuno, Do-Youn Oh, Andree Rorive, Hatem Soliman, Shunji Takahashi, Tanios Bekaii-Saab, Mark E Burkard, Ki Y Chung, Philip R Debruyne, Jenny R Fox, Valentina Gambardella, Marta Gil-Martin, Erika P Hamilton, Bradley J Monk, Yoshiaki Nakamura, Danny Nguyen, David M O'Malley, Alexander B Olawaiye, Bhavana Pothuri, Martin Reck, Kazuki Sudo, Yu Sunakawa, Cedric Van Marcke, Evan Y Yu, Jorge Ramos, Sherry Tan, Mark Bieda, Thomas E Stinchcombe, Paula R Pohlmann*. Nat Med 2025. doi: 10.1038/s41591-024-03462-0.

What's new from IEO Researchers?

Migliorare la risposta al trattamento delle pazienti con tumore al seno ER+/HER2- con l'immunoterapia - risultati di uno studio clinico di fase III.

Ad oggi l'approccio terapeutico standard per il trattamento delle pazienti con tumore al seno ER+/HER2-, negli stadi iniziali della malattia, prevede la chemioterapia (in un contesto neoadiuvante o adiuvante) e una terapia endocrina prolungata, in alcuni casi in combinazione con la terapia molecolare. La risposta di queste pazienti al trattamento è però molto variabile.

Dato che con l'immunoterapia si sono ottenuti risultati notevoli nelle pazienti con tumore al seno triplo-negativo (TNBC) e alcune pazienti ER+/HER2- presentano –come nel TNBC– un'abbondanza di cellule immunitarie all'interno della massa tumorale, i ricercatori hanno ipotizzato che l'immunoterapia (nello specifico, nivolumab, farmaco contro la proteina PD1 espressa dalle cellule immunitarie, interferendo con il suo legame con la proteina PDL1 sulle cellule tumorali), promuovendo l'attività immunitaria antitumorale, potrebbe migliorare la risposta delle pazienti all'attuale terapia neoadiuvante standard. Quindi, nel contesto di uno studio clinico di fase III, i ricercatori, tra cui Giuseppe Curigliano –vice-direttore scientifico di IEO, direttore della divisione di Sviluppo Nuovi Farmaci per Terapie Innovative e co-direttore del Programma Nuovi Farmaci– hanno valutato sicurezza ed efficacia della somministrazione di immunoterapia (nivolumab) insieme alla chemioterapia (antracicline e taxani), nel setting neoadiuvante, per il trattamento delle pazienti ER+/HER2- negli stadi iniziali della malattia.

La **terapia neoadiuvante** è definita come la somministrazione di un data terapia *prima* del trattamento principale. Ad esempio, prima dell'intervento chirurgico, la terapia neoadiuvante viene somministrata al fine di ridurre le dimensioni del tumore e permettere di ottenere un esito clinico migliore. Al contrario, la **terapia adiuvante** viene somministrata *dopo* il trattamento principale (ad esempio dopo l'intervento chirurgico) al fine di ridurre le probabilità di recidiva.

Nivolumab. Anticorpo monoclonale che, legandosi in maniera specifica alla proteina PD1 espressa sulle cellule immunitarie, impedisce l'interazione di PD1 con la proteina PDL1 sulle cellule tumorali. Interferendo con l'interazione tra PD1 e PDL1, nivolumab interferisce con un meccanismo fisiologico normalmente coinvolto nel "frenare", e quindi controllare, l'attività eccessiva del sistema immunitario contro le cellule che esprimono PDL1. Rimuovendo questa inibizione, le cellule immunitarie risultano più attive e quindi maggiormente in grado di eliminare le cellule tumorali che esprimono PDL1.

I loro risultati forniscono informazioni fondamentali per il trattamento di questo sottotipo di tumore al seno per il quale sono stati fatti numerosi tentativi –ad oggi con scarso successo– per poter migliorare la risposta alla terapia. L'aggiunta dell'immunoterapico nivolumab alla chemioterapia ha migliorato in maniera significativa la risposta delle pazienti. Gli effetti di questo trattamento combinato erano particolarmente evidenti nelle pazienti con tumori PDL1-positivi e in quelle con un numero più elevato di cellule immunitarie intratumorali; per queste pazienti, questo tipo di trattamento potrebbe rappresentare il nuovo approccio terapeutico standard. Sebbene i risultati non siano sufficienti a trarre delle conclusioni riguardo agli effetti sulla sopravvivenza, sulla base dei risultati di studi precedenti è altamente probabile che la maggiore risposta alla terapia correli con una maggiore sopravvivenza (*event-free survival*),

incoraggiando fortemente studi ulteriori volti a verificare se la maggiore risposta alla terapia possa tradursi in una maggiore sopravvivenza in tutte le pazienti ER+/HER2- o solo nella popolazione PDL1-positiva.

TELL ME MORE!

Le pazienti sono state arruolate in 221 diversi centri di cura in 31 paesi. Un primo gruppo di 257 pazienti ha ricevuto, in un contesto neoadiuvante, chemioterapia e immunoterapia, mentre un secondo gruppo di 253 pazienti ha ricevuto chemioterapia e un placebo.

Efficacia. La somministrazione di immunoterapia insieme alla chemioterapia determinava una migliore risposta clinica: gli autori hanno osservato una più elevata percentuale di pazienti che mostravano, alla fine del trattamento, una risposta clinica completa o malattia residua minima. La sopravvivenza (*event-free survival*) appariva simile nei due gruppi di pazienti, ma la durata del follow-up non era sufficiente a trarre conclusioni statisticamente rilevanti.

Dato che nivolumab colpisce l'asse PD1/PDL1, gli autori hanno valutato la possibilità di identificare le pazienti con buone probabilità di rispondere a questo trattamento sulla base del grado di infiltrazione immunitaria intratumorale e dei livelli di espressione della proteina PDL1. L'effetto era più evidente nei tumori PDL1-positivi e che mostravano un numero più elevato di cellule immunitarie intratumorali rispetto ai tumori PDL1-negativi e caratterizzati da una scarsa infiltrazione del tumore da parte delle cellule immunitarie, ma studi approfonditi sull'efficacia del trattamento in relazione all'espressione dei biomarcatori hanno rivelato che anche le pazienti con tumori PDL1-negativi o privi di cellule immunitarie intratumorali traevano alcuni benefici.

Sicurezza. Il profilo di sicurezza era in linea con quanto già noto e non ha impedito di sottoporre le pazienti a chirurgia dopo il trattamento neoadiuvante: nel complesso, si manifestavano eventi avversi sia nelle pazienti che ricevevano chemioterapia e immunoterapia che in quelle che ricevevano chemioterapia e un placebo; tuttavia, eventi avversi severi, associati al trattamento, che in alcuni casi hanno determinato l'interruzione del trattamento, erano più frequenti nel gruppo di pazienti sottoposte a terapia di combinazione (che nel placebo). In alcune pazienti, è stato necessario somministrare dei farmaci immunomodulatori.

Referenza. Neoadjuvant nivolumab and chemotherapy in early estrogen receptor-positive breast cancer: a randomized phase 3 trial. Sherene Loi, Roberto Salgado, Giuseppe Curigliano, Roberto Iván Romero Díaz, Suzette Delaloge, Carlos Ignacio Rojas García, Marleen Kok, Cristina Saura, Nadia Harbeck, Elizabeth A Mittendorf, Denise A Yardley, Alberto Suárez Zaizar, Facundo Rufino Caminos, Andrei Ungureanu, Joaquin G Reinoso-Toledo, Valentina Guarneri, Daniel Egle, Felipe Ades, Misena Pacius, Aparna Chhibber, Rajalakshmi Chandra, Raheel Nathani, Thomas Spires, Jenny Qun Wu, Lajos Pusztai, Heather McArthur. Nat Med 2025. doi: 10.1038/s41591-024-03414-8.

What's new from IEO Researchers?

Combinare immunoterapia e chemioterapia per il trattamento del tumore dell'endometrio.

Nicoletta Colombo

Studi preclinici suggeriscono che la somministrazione combinata di immunoterapia e chemioterapia potrebbe offrire un approccio sinergico nel trattamento del tumore dell'endometrio. Inoltre, studi clinici hanno mostrato l'efficacia del trattamento combinato con immunoterapia anti-PDL1 e chemioterapia nel tumore della cervice, nel tumore al seno triplo negativo e nel tumore polmonare. Tuttavia, ad oggi i dati disponibili riguardo alla potenziale efficacia di questa terapia combinata nel tumore dell'endometrio sono limitati e in questo tumore il trattamento standard di prima linea è rappresentato dalla chemioterapia (carboplatin–paclitaxel), con una sopravvivenza delle pazienti di circa tre mesi.
Nel contesto dello studio clinico internazionale di fase III AtTEnd diretto da Nicoletta Colombo -Direttrice del Programma Ginecologia di IEO e Professore Associato presso l'Università degli Studi di Milano-Bicocca-, gli autori hanno mostrato efficacia e sicurezza della terapia combinata con immunoterapia anti-PDL1 (ovvero, l'inibitore del checkpoint immunitario atezolizumab) e chemioterapia (carboplatin/paclitaxel) per il trattamento del tumore endometriale avanzato/recidivo.
E' interessante sottolineare che questa terapia di combinazione ha mostrato un'efficacia significativa nei pazienti con difetti nel sistema di riparo del DNA danneggiato (*mismatch repair*, MMR). Questo approccio terapeutico ha migliorato in maniera significativa *progression-free survival e overall survival*, proponendosi come nuovo standard di cura in questi pazienti.

TELL ME MORE!

Lo studio AtTEnd ha arruolato 551 pazienti in 89 centri di cura in 11 diversi paesi. Le pazienti sono state casualmente assegnate a ricevere chemioterapia (carboplatino e paclitaxel) e immunoterapia anti-PDL1 o chemioterapia ed un placebo. AtTEnd è il primo grande studio di fase III, con un lungo follow-up (mediano: 28.3 months), che mostra una migliore *progression-free survival* e *overall survival* del trattamento combinato con atezolizumab e chemioterapia in pazienti con tumore endometriale avanzato/recidivo. La maggiore efficacia del trattamento osservata nel sottogruppo di pazienti con difetti del sistema (MMR) di riparo del DNA è in linea con studi precedenti che mostrano l'efficacia del trattamento combinato con immunoterapia anti-PD1 e chemioterapia in questi pazienti. Infatti, un sistema MMR malfunzionante potrebbe determinare un aumento del numero di mutazioni, generando neoantigeni che, riconosciuti dal sistema immunitario, ne promuoverebbero l'attività antitumorale. Tuttavia, ulteriori analisi, nei diversi sottogruppi di pazienti, sono fondamentali al fine di considerare fattori aggiuntivi, come la diversa etnia e il microbiota (il cui ruolo nel modulare l'efficacia dell'immunoterapia negli ultimi anni si sta rapidamente consolidando), prima di trarre delle conclusioni definitive riguardo all'efficacia del trattamento combinato nelle pazienti con tumori caratterizzati da un sistema MMR funzionante.

Referenza. Atezolizumab and chemotherapy for advanced or recurrent endometrial cancer (AtTEnd): a randomised, double-blind, placebo-controlled, phase 3 trial. *Nicoletta Colombo, Elena Biagioli, Kenichi Harano, Francesca Galli, Emma Hudson, Yoland Antill, Chel Hun Choi, Manuela Rabaglio, Frederic Marmé, Christian Marth, Gabriella Parma, Lorena Fariñas-Madrid, Shin Nishio, Karen Allan, Yeh Chen Lee, Elisa Piovano, Beatriz Pardo, Satoshi Nakagawa, John McQueen, Claudio Zamagni, Luis Manso, Kazuhiro Takehara, Giulia Tasca, Annamaria Ferrero, Germana Tognon, Andrea Alberto Lissoni, Mariacristina Petrella, Maria Elena Laudani, Eliana Rulli, Sara Uggeri, M Pilar Barretina Ginesta; AtTEnd study group*. Lancet Oncol 2024. doi: 10.1016/S1470-2045(24)00334-6.

What's new from IEO Researchers?

In che modo il microbiota intestinale modula la risposta dei pazienti ad immunoterapia?

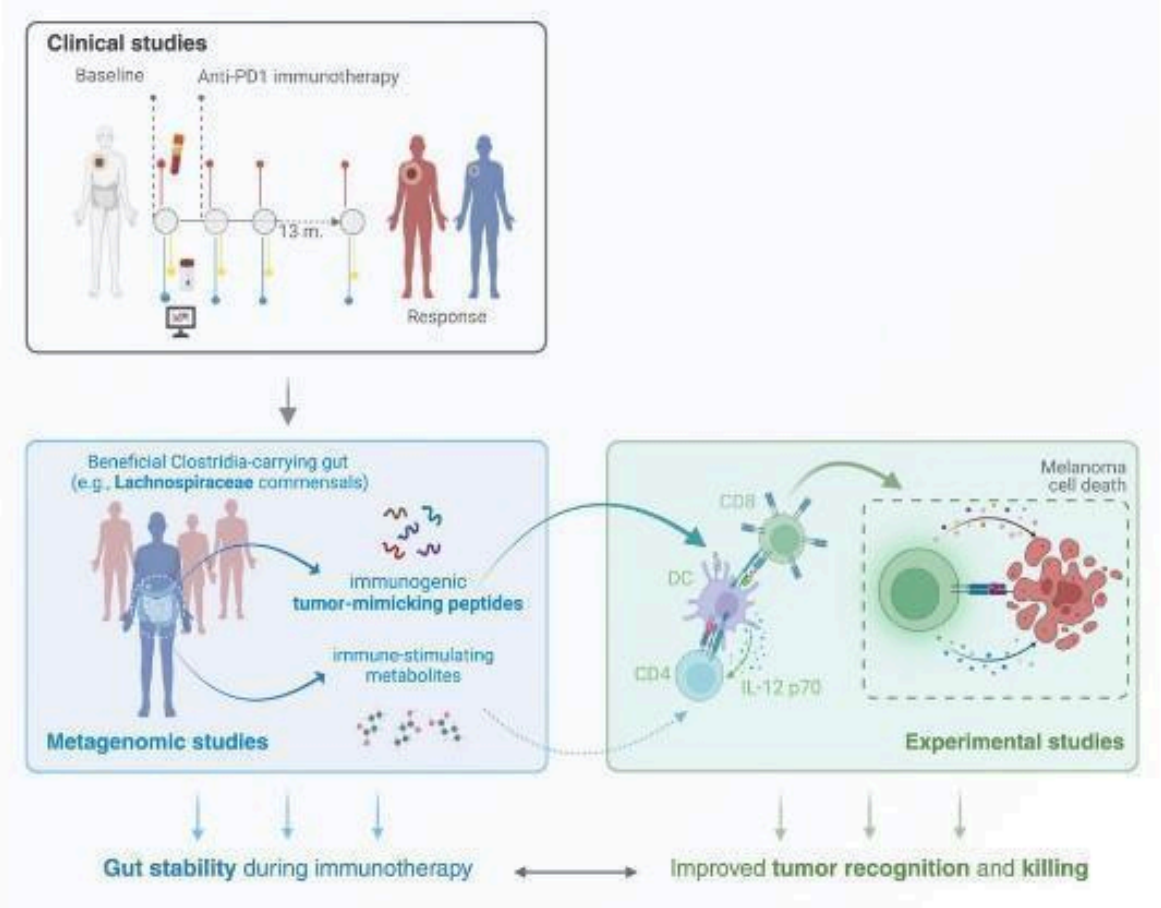


Figure from Macandog, Catozzi et al., Cell Host & Microbe 2024 (an open access article under the CC BY NC ND licence.)

Sebbene la terapia con inibitori dei checkpoint immunitari (ICI) abbia migliorato in maniera significativa la cura dei pazienti con melanoma, molti di loro non rispondono al trattamento. Studi precedenti hanno mostrato una diversa composizione del microbiota intestinale dei pazienti precedentemente trattati che hanno risposto al trattamento (responder) rispetto ai pazienti che non hanno risposto alla terapia (non-responder) e un ruolo modulatorio del microbiota intestinale, che è stato mostrato aumentare l'efficacia della terapia in pazienti che hanno ricevuto trapianto di microbiota fecale (FMT) da responder o donatori sani (e.g. Newsletter n.1), in alcuni casi determinando un effetto della terapia anche in pazienti refrattari.

Al fine di comprendere i meccanismi attraverso cui il microbiota intestinale modula la risposta alla terapia, in un articolo recente di Macandog, Catozzi et al., gli autori, supervisionati da Luigi Nezi –Group Leader del dipartimento di oncologia sperimentale di IEO–, hanno caratterizzato il microbioma intestinale dei pazienti con melanoma prima dell'inizio del trattamento e durante la terapia con anti-PD1 e hanno analizzato i

corrispondenti campioni di sangue –per descrivere gli effetti a livello sistemico–, mostrando che un microbiota intestinale che rimane "stabile" –in termini di composizione– nel tempo durante la terapia

Nezi Lab

correla con la risposta alla terapia. Inoltre, i ricercatori hanno identificato dei geni microbici –stabilmente presenti, dall'inizio fino all'uscita dei pazienti dallo studio– dotati di uno straordinario potere prognostico che, per via della loro presenza nei pazienti già prima della terapia, consentivano di distinguere i pazienti che mostravano una risposta completa alla terapia (responder completi, CR) da coloro che non mostravano una risposta completa (*non-complete responder*, nonCR), in questo studio e in altre coorti di pazienti. Infine, gli autori hanno dimostrato sperimentalmente che alcuni peptidi batterici derivanti da questi geni microbici sono estremamente simili ad alcune proteine tumorali e possono essere utilizzati per potenziare la risposta ad immunoterapia.

Questo lavoro, quindi, da un lato dimostra l'importanza di un'analisi longitudinale del microbioma intestinale, che infatti ha permesso di identificare delle specie del microbiota intestinale associate con la risposta alla terapia, laddove la sola analisi prima della terapia non ha fornito la solidità necessaria ai dati. Inoltre, l'analisi contemporanea di microbioma e campioni di sangue ha permesso di correlare microbioma, effetti sistemici a livello del sistema immunitario e risposta alla terapia. Dall'altro, questo studio ha identificato dei fattori (peptidi) del microbioma in grado di potenziare la risposta alla terapia, approfondendo i dettagli meccanicistici del legame microbioma/sistema immunitario, alla base dell'interazione reciproca tra i due.

TELL ME MORE!

Prima di tutto, gli autori hanno analizzato in che modo la somministrazione di ICI modificava la composizione del microbiota intestinale e come questo fosse collegato con la risposta alla terapia

e hanno caratterizzato campioni fecali di pazienti con melanoma in stadio avanzato, prima e durante la terapia con ICI, a diversi time point. Hanno quindi ipotizzato che le specie prevalenti nei diversi campioni raccolti a diversi time point erano molto probabilmente biologicamente rilevanti. Con questo presupposto, hanno scoperto che mentre le specie diversamente abbondanti nei CR rispetto ai nonCR non mantenevano la stessa abbondanza ai diversi time point (cioè non necessariamente una specie che era più abbondante nei CR che nei nonCR al primo time point rimaneva più abbondante al time point successivo e così via), le specie prevalenti nei CR rimanevano prevalenti ai differenti time point analizzati, suggerendo la loro rilevanza biologica. Inoltre, anche in altre (4 delle 9 analizzate) coorti di pazienti, in cui studi precedenti hanno riscontrato una scarsa riproducibilità delle signature basate sul microbioma, le stesse specie associate a CR identificate in questo lavoro conservavano la loro maggiore presenza nei responder, sia prima che durante la terapia.

Dato che il microbiota intestinale influenza il sistema immunitario e il sistema immunitario influenza il microbiota, dopo aver analizzato il microbiota hanno analizzato i campioni di sangue (ovvero la conta dei globuli bianchi e la quantificazione dei fattori infiammatori solubili) raccolti durante la terapia, così da approfondire gli effetti sistemici, a livello del sistema immunitario, e metterli in relazione con le caratteristiche del microbioma intestinale. Il rapporto neutrofili/linfociti (NLR) era basso (cioè, bassa conta dei neutrofili e alta conta dei linfociti) durante la terapia nei CR rispetto ai nonCR. Inoltre, l'analisi delle molecole infiammatorie solubili, prima e durante la terapia, ha rivelato che la presenza nel sangue di una specifica molecola infiammatoria (IL-12p70) era associata con CR, mentre quattro altre (CX3CL1/FRACTALKINE, IL-7, IL-8, HGF) erano associate con nonCR. I livelli di queste molecole infiammatorie erano anche associati con NLR. Inoltre, i livelli della citochina associata a CR correlavano con le specie del microbioma che rimanevano stabili ai diversi time point nei CR. Al contrario, non vi era associazione tra le quattro citochine associate con i nonCR e le specie del microbioma, evidenziando l'elevata variabilità della composizione del microbiota dei nonCR, diversamente dal microbiota dei CR.

Nel complesso, questi risultati dimostrano la rilevanza clinica della caratterizzazione longitudinale del microbiota intestinale, che ha permesso la stratificazione dei pazienti in responder (completi) e non, cosa che invece non è stata possibile con precedenti signature del microbiota ottenute solo attraverso l'analisi della composizione del microbiota ad un solo ad un time point, ovvero prima della terapia. L'analisi funzionale ha rivelato alcuni pathway microbici maggiormente presenti, in maniera stabile sia prima che durante la terapia, nel microbiota dei CR (ovvero i pathway attivi nelle specie sovra-rappresentate nel microbiota intestinale dei CR), come quelli coinvolti nell'assemblaggio dei flagelli e nella chemiotassi dei batteri, così come nel metabolismo di zuccheri e amido. La situazione era molto meno definita nei nonCR. E' interessante sottolineare che le famiglie di geni associate con questi pathway batterici erano predittori migliori della risposta a ICI delle specie batteriche stesse, permettendo di distinguere CR e nonCR anche in altre coorti di pazienti, prima della terapia. Data la rilevanza clinica della loro scoperta, gli autori hanno approfondito i meccanismi alla base delle differenze identificate, a livello del microbiota, tra i CR e i nonCR. I geni della flagellina (coinvolti nell'assemblaggio dei flagelli) erano i più sovra-rappresentati nel microbiota intestinale dei CR. Queste proteine sono note essere coinvolte nella modulazione del sistema immunitario. In particolare, gli autori hanno osservato che tra le proteine di flagellina della specie Lachnospiraceae (FLach), alcune (tre) erano molto simili (sia in termini di sequenza che di struttura 3D prevista in silico) ad antigeni associati al tumore (TAAs) del melanoma. Questi TAAs erano preferenzialmente espressi sui tumori dei CR rispetto a quelli dei nonCR. Hanno approfondito la relazione tra le proteine di FLach

NLR, ovvero il rapporto tra la conta di neutrofili e linfociti nel sangue periferico, è generalmente considerato un marcatore di malattia, la manifestazione di uno stato infiammatorio, in diverse condizioni patologiche, tra cui infezioni e cancro. Diversi studi suggeriscono che il NLR potrebbe essere usato come marcatore prognostico in diversi tipi di tumore, proponendone un eventuale utilizzo in ambito clinico.

e il sistema immunitario e hanno mostrato una maggiore reattività dei linfociti T contro i peptidi di FLach isolati (prima della terapia con ICI) dal sangue dei pazienti CR con melanoma rispetto ai nonCR. Inoltre, i peptidi di FLach inducevano una maggiore espansione *in vitro* dei linfociti intratumorali (TIL) isolati dal melanoma, dimostrando la presenza di un'immunità intra-tumorale FLach-specifica che potrebbe essere sfruttata a fini terapeutici. Infatti, i TIL espansi *in vitro* con i peptidi di FLach esibivano una maggiore capacità, negli organoidi, di uccidere le cellule tumorali, dimostrando che questi peptidi erano in grado di potenziare la risposta immunitaria antitumorale, antigene-specifica contro il melanoma.

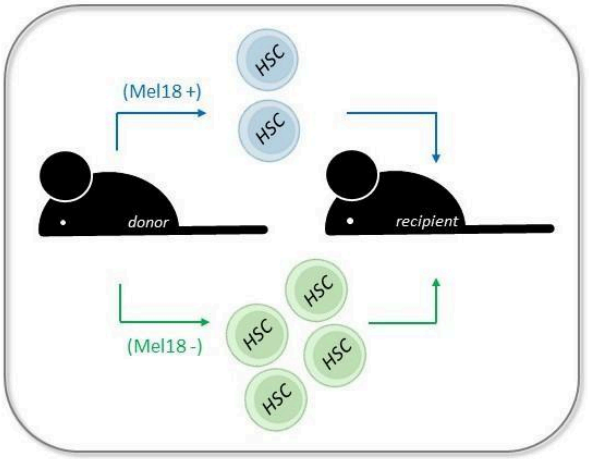
Quindi, se da un lato è stato precedentemente proposto che il microbiota intestinale influenzi il sistema immunitario attraverso il rilascio di metaboliti, questi risultati suggeriscono fortemente che potrebbe accadere anche attraverso la modulazione diretta del sistema immunitario da parte delle proteine di batteri intestinali, come le flagelline. Sono attualmente in corso degli studi per sfruttare i peptidi di FLach per sviluppare strumenti predittivi e terapeutici innovativi, al fine di migliorare le prospettive dei pazienti con melanoma e, potenzialmente, altri tumori solidi.

Referenza. Longitudinal analysis of the gut microbiota during anti-PD-1 therapy reveals stable microbial features of response in melanoma patients. *Angeli D G Macandog, Carlotta Catozzi, Mariaelena Capone, Amir Nabinejad, Padma P Nanaware, Shujing Liu, Smita Vinjamuri, Johanna A Stunnenberg, Serena Galiè, Maria Giovanna Jodice, Francesca Montani, Federica Armanini, Ester Cassano, Gabriele Madonna, Domenico Mallardo, Benedetta Mazzi, Salvatore Pece, Maria Tagliamonte, Vito Vanella, Massimo Barberis, Pier F Ferrucci, Christian U Blank, Marlene Bouvier, Miles C Andrews, Xiaowei Xu, Laura Santambrogio, Nicola Segata, Luigi Buonaguro, Emilia Cocorocchio, Paolo A Ascierto, Teresa Manzo, Luigi Nezi*. Cell Host Microbe 2024. doi: 10.1016/j.chom.2024.10.006.

What's new from IEO Researchers?

Colpire il complesso Polycomb per aumentare la proliferazione delle cellule staminali ematopoietiche prima di un trapianto. Una nuova possibile strategia?

Tutte le cellule del sangue derivano da una sottopopolazione di cellule chiamate cellule staminali ematopoietiche (HSC). Queste cellule assicurano l'omeostasi del sangue (ovvero il corretto equilibrio, in ogni momento, tra le diverse componenti cellulari del sangue) nel corso della vita e giocano un ruolo fondamentale durante il trapianto di cellule staminali ematopoietiche (HSCT). Queste cellule sono infatti dotate della cosiddetta capacità di self-renewal, ovvero la capacità, una volta trapiantate in un paziente, di proliferare e differenziarsi praticamente in tutti i tipi cellulari del sangue. I meccanismi alla base della self-renewal delle HSC non sono però completamente chiari e una comprensione approfondita potrebbe permettere di interferire in maniera precisa con questi meccanismi e migliorare l'efficacia del trapianto.



In un articolo recente, i ricercatori –tra cui Diego Pasini, Group Leader del dipartimento di oncologia sperimentale di IEO, nel contesto di una collaborazione con il gruppo di Yan Liu alla Northwestern University (Chicago)– hanno dimostrato il ruolo chiave delle proteine del complesso Polycomb –proteine fondamentali nella fisiologia cellulare, che agiscono reprimendo l'espressione di specifici geni quando necessario (Tamburri et al., Mol Cell 2024. Newsletter n.5)– nella self-renewal delle HSC. In particolare, gli autori hanno dimostrato che la subunità Mel18 del complesso Polycomb inibisce la capacità di self-renewal delle HSC, mentre la sua assenza determina un aumento della self-renewal delle HSC, promuovendone la proliferazione, sia in saggi *in vitro* che in modelli preclinici *in vivo*.

Le loro scoperte appaiono clinicamente rilevanti nel contesto del HSCT. Infatti, sebbene questo approccio sia ampiamente utilizzato clinicamente per il trattamento dei pazienti con leucemia, la capacità limitata, con i metodi attualmente disponibili, di espandere le HSC isolate da un donatore prima del trapianto in un ricevente costituisce una notevole difficoltà. Questo lavoro propone di approfondire la possibilità di utilizzare un approccio che sfrutta l'attività repressiva del complesso Polycomb, e in particolare della subunità Mel18, per migliorare l'espansione *ex vivo* delle HSC raccolte da un donatore: la rimozione –o inibizione– strettamente controllata, di Mel18 potrebbe infatti permettere di aumentare l'espansione delle HSC prima di un trapianto, facilitando così l'intero processo di manifattura collegato al HSCT e riducendo i costi associati.

TELL ME MORE!

La proteina del gruppo Polycomb Bmi1 è nota per il suo ruolo fondamentale nella self-renewal delle HSC: la perdita di Bmi1 danneggia la capacità di self-renewal delle HSC, mentre la sovraespressione di Bmi1 determina un aumento della self-renewal delle HSC. Sfruttando modelli murini condizionali privi di un'altra subunità del complesso Polycomb, Mel18, nel sistema ematopoietico, gli autori hanno osservato che la perdita di Mel18 alterava la composizione del sangue (aumentando, ad esempio, il numero di monociti e riducendo il numero di neutrofili,

piastrine, e globuli rossi) e il numero di HSC (determinando un aumento di LT-HSC, MPP e Lin-, senza influenzare le cellule ST-HSCs, cKIT+ cells). Hanno infatti osservato che la perdita di Mel18 promuoveva la self-renewal delle HSC, sia in saggi *in vitro* (colony forming assay) che *in vivo* (aumentando l'attecchimento delle cellule HSC prive di Mel18 negli animali riceventi, irradiati), e la progressione del ciclo cellulare. L'aumentata self-renewal delle HSC prive di Mel18 era associata a cambiamenti dell'espressione genica, tra cui l'espressione di geni collegati alle HSC (come Hoxb4, la cui espressione ectopica è nota aumentare l'espansione *ex vivo* delle HSC). L'alterato profilo di espressione genica indotto dalla perdita di Mel18 era dovuto ad un cambiamento nell'accessibilità della cromatina, che infine determinava un aumento della self-renewal delle HSC. La ri-espressione ectopica di Mel18 riduceva l'espressione di Hoxb4 e la self renewal, indicando che Mel18 regolava negativamente il mantenimento delle HSC e quindi la sua assenza promuoveva l'espansione delle HSC. Inoltre, l'assenza di Mel18 causava la mancata repressione di geni del ciclo cellulare –come le chinasi ciclina-dipendenti, E2F1, Myc– promuovendo così la progressione del ciclo cellulare.

La perdita di Mel18 influenzava geni differenti rispetto a quelli regolati da Bmi1, indicando che le due proteine ricoprono ruoli distinti e agiscono attraverso la regolazione di geni diversi. Dal punto di vista del meccanismo, Mel18 –che è parte del complesso Polycomb1– funziona reprimendo l'espressione genica attraverso l'aggiunta di molecole di ubiquitina sulle proteine istoniche e, infatti, i livelli di ubiquitinazione a livello dell'istone 2 (H2AK119ub1) erano alterati nelle cellule prive di Mel18. In particolare, tra i geni che mostravano una ridotta ubiquitinazione a livello dell'istone 2 in seguito alla perdita di Mel18, vi erano i geni associati al ciclo cellulare.

Referenza. Polycomb group protein Mel18 inhibits hematopoietic stem cell self-renewal through repressing the transcription of self-renewal and proliferation genes. *Wenjie Cai, Xicheng Liu, Sergio Barajas, Shiyu Xiao, Sasidhar Vemula, Hongxia Chen, Yuxia Yang, Christopher Bochers, Danielle Henley, Sheng Liu, Yuzhi Jia, Michelle Hong, Tiffany M Mays, Maegan L Capitano, Huiping Liu, Peng Ji, Zhonghua Gao, Diego Pasini, Jun Wan, Feng Yue, Leonidas C Platanias, Rongwen Xi, Sisi Chen, Yan Liu.* Leukemia 2025. doi: 10.1038/s41375-024-02462-w.

E se misurazioni "imprecise" (*biased*) portassero ad un'interpretazione sbagliata?
Testare in maniera non equilibrata condiziona i risultati degli studi sulle interazioni proteina-proteina.

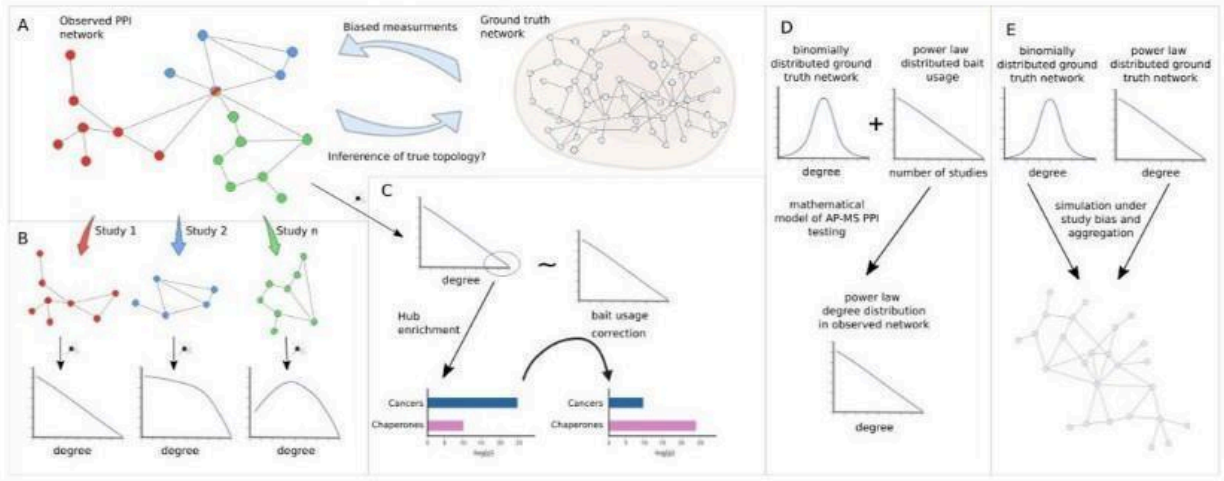


Figure from Blumenthal, Lucchetta et al., eLife2024 (an open access article under the CC BY licence)

Uno stato patologico può essere dovuto ad alterazioni nei livelli di espressione o nella sequenza di una proteina, o ad interazioni anomale di una proteina con altre proteine. Ad esempio, nella leucemia, le mutazioni nella proteina NPM1 inducono l'interazione anomala –assente in un contesto fisiologico– con la proteina ARF, che causa la delocalizzazione e la degradazione di NPM1, lasciando a sua volta la proteina MDM2 libera di indurre ubiquitinazione e degradazione di p53, prevenendo così la morte cellulare dipendente da p53 in risposta a segnali di stress e quindi la trasformazione maligna. Questo meccanismo è un esempio di tumorigenesi indotta da cambiamenti nell'interazione proteina-proteina.
Gli strumenti computazionali sono sempre più spesso utilizzati nella ricerca biomedica e, ad esempio, possono essere sfruttati per caratterizzare le interazioni proteina-proteina e predire il coinvolgimento di una data proteina in una malattia. Un lavoro precedente, che ha coinvolto i ricercatori IEO, ha riportato lo sviluppo di un'applicazione web –Robust-web– per identificare, all'interno di una rete di interazioni proteina-proteina, dei sottogruppi di interazioni tra proteine con specifiche caratteristiche, come ad esempio l'importanza in un dato stato patologico (Sarkar et al., Bioinformatics 2023. Newsletter n.1).
Per descrivere la probabilità delle singole proteine di formare uno specifico numero di interazioni, i ricercatori generalmente utilizzano modelli matematici che si basano sulla *Power Law*; ovvero, una funzione

matematica che descrive il grado di distribuzione della rete di proteine (in altre parole, la *Power Law* fitta il numero di interazioni che ogni proteina tende a formare.

In un articolo recente di Blumenthal, Lucchetta et al., i risultati della ricerca che ha coinvolto Martin Schaefer -Group Leader al dipartimento di oncologia sperimentale di IEO-, mostrano però che solo alcune (meno del 30%) delle reti di interazioni proteina-proteina potrebbero di fatto essere descritte dalla *Power Law* (cioè, solo in alcune reti di proteine la *Power Law* di fatto approssima in maniera accurata il grado di distribuzione). Ciò è verosimilmente dovuto al fatto che le reti di interazioni proteina-proteina ottenute aggregando i dati derivanti da studi diversi sono profondamente influenzate dalle proteine più frequentemente studiate (Newsletter n.1), introducendo un *bias* (di fatto, un errore), insieme all'elevata frequenza di errori associata con i metodi per rilevare l'interazione proteina-proteina. In questo studio, gli autori dimostrano matematicamente ed empiricamente che, per via di questo *bias*, anche le reti che non potrebbero essere descritte dalla *Power Law* sembra che lo siano. Di conseguenza, le descrizioni delle reti di interazione tra proteine possono essere sbagliate: potrebbe sembrare che alcune proteine interagiscano con altre proteine quando invece non è così e, viceversa, alcune interazioni che in realtà si verificano all'interno della cellula potrebbero non essere rilevate. Dal punto di vista biologico, causando l'identificazione erronea di interazioni tra proteine, alcune proteine potrebbero risultare associate con una malattia quando invece non lo sono. Correggendo invece questo *bias*, le reti di proteine non possono essere più descritte dalla *Power Law*.

E' stato accertato l'effetto di un *bias*. Può essere corretto?

"Un modo per evitare questo problema –suggerisce Martin Schaefer– potrebbe essere quello di studiare le interazioni proteina-proteina in maniera sistematica, senza una ipotesi/conoscenza a priori dell'importanza di una data proteina in una cellula (su questo principio di basa il progetto HuRI, attualmente in corso; Luck et al., Nature 2020). D'altro canto, il *bias* potrebbe venire amplificato dall'aggregazione dei dati dei singoli studi e le analisi effettuate su dati non aggregati, normalizzando il grado di distribuzione, o prendendo in considerazione nell'algoritmo la frequenza con cui le proteine sono analizzate, potrebbe contribuire a ridurre il *bias* e quindi consentire una migliore comprensione delle reti cellulari senza dare troppo peso al ruolo delle proteine più frequentemente studiate".

Qual è la conseguenza di questa scoperta per gli studi futuri in ambito biologico? Innanzi tutto, questi risultati suggeriscono cautela nell'interpretazione dei dati di interattomica. Infatti, il *bias* menzionato appare particolarmente rilevante nel contesto della ricerca sul cancro. L'interattoma si riferisce all'insieme delle interazioni proteina-proteina che avvengono all'interno di una cellula e rivelare queste interazioni è fondamentale per comprendere il comportamento di una cellula. Ogni proteina svolge la sua funzione non da sola all'interno di una cellula, ma come parte di una rete. L'identificazione di anomalie nelle interazioni tra proteine –cioè differenze rispetto allo stato fisiologico– può rivelare nuovi meccanismi di malattia con cui poter potenzialmente interferire farmacologicamente.

Inoltre, queste scoperte indicano che il crescente utilizzo delle proprietà della *Power Law* per le reti biologiche potrebbe non essere spiegato biologicamente, come è stato proposto precedentemente, dal "modello di duplicazione genica" (modello secondo cui la *Power Law* descrive la rete di interazioni proteina-proteina considerando che le proteine originate da una duplicazione genica potrebbero mantenere lo stesso set di interazioni con altre proteine, mentre le mutazioni potrebbero permettere nuove interazioni).

Referenza. Emergence of power law distributions in protein-protein interaction networks through study bias. *David B Blumenthal #, Marta Lucchetta #, Linda Kleist, Sándor P Fekete, Markus List, Martin H Schaefer*. Elife 2024. doi: 10.7554/eLife.99951.

News, initiatives and events from the IEO world!

Due progetti di ricerca IEO premiati al Seed4Innovation.

Due progetti di ricerca sviluppati presso l'IRCCS Istituto Europeo di Oncologia (IEO) sono stati premiati nella quarta edizione del programma Seed4Innovation, il programma di innovazione promosso da Fondazione UNIMI e Università degli Studi di Milano.

Il progetto STRIVE, coordinato dal prof. Stefano Santaguida – group leader IEO e professore associato dell'Università degli Studi di Milano –, ha ricevuto il Grant Proof of Concept di Università degli Studi di Milano, e si pone come obiettivo quello di sviluppare nuove terapie per il tumore al seno, attraverso l'identificazione di specifici marcatori di superficie presenti sulle cellule tumorali.

Il progetto EPIKIN, sviluppato dal team guidato dal dott. Gioacchino Natoli – group leader IEO -, con il contributo di Francesco Gualdrini e Sara Polletti, ha ottenuto il Grant Proof of Concept di Camera di Commercio Milano Monza Brianza Lodi, ed è una piattaforma innovativa per la profilazione genomica degli inibitori delle chinasi migliorando l'avanzamento e lo sviluppo clinico di queste molecole e aprendo nuove opportunità terapeutiche.

IEO-TT, l'Ufficio di Trasferimento Tecnologico di IEO, guidato da Marzia Fumagalli e con il contributo operativo di Claudia Iavarone, Senior Specialist presso IEO-TT, ha affiancato con entusiasmo i team di ricerca in ogni fase del programma Seed4Innovation. L'ufficio continuerà a offrire il proprio supporto nelle fasi successive di accelerazione, con l'obiettivo di trasformare queste idee innovative in realtà concrete.

Un ringraziamento speciale alla Fondazione UNIMI, all'Università degli Studi di Milano e alla rete di mentors coordinata da Antonio Alessandrino, che hanno accompagnato i ricercatori in questo percorso, ed in particolare i mentors Lavinia Capuana, Fabrizio Bacchi, Paolo Luperto e Gianluca Sferrazza che hanno svolto un ruolo cruciale nel rafforzare la visione e il potenziale impatto dei progetti IEO.

News, initiatives and events from the IEO world!

Giornata dell'Innovazione 2024.

Si è tenuta il 19 dicembre 2024, presso l'IRCCS Istituto Europeo di Oncologia (IEO), la Giornata dell'Innovazione 2024, un evento organizzato per celebrare 30 anni di ricerca, progresso e impegno dell'istituto nel miglioramento della salute e del benessere.

Responsabile scientifico della giornata la dott.ssa Marzia Fumagalli, responsabile di IEO-TT, Ufficio di Trasferimento Tecnologico di IEO, che ha moderato i lavori con la partecipazione di professionisti del settore, ricercatori e innovatori che hanno discusso le sfide attuali, le nuove opportunità e le tecnologie emergenti che stanno plasmando il futuro della medicina in oncologia, dalle soluzioni digitali alla personalizzazione delle cure.

La giornata è iniziata con i saluti istituzionali dell'Ing. Mauro Melis, Amministratore Delegato di IEO, seguiti da un discorso di benvenuto del Direttore Scientifico prof. Roberto Orecchia e del Direttore del Dipartimento di Oncologia Sperimentale prof. Pier Giuseppe Pelicci, che hanno sottolineato come l'innovazione sia il motore indispensabile per il nostro futuro. Francesco Cerruti, Direttore Generale dell'Italian Tech Alliance, ha aperto i lavori parlando delle sfide e delle opportunità dell'innovazione in Italia con una prospettiva fiduciosa e positiva per i prossimi anni. Due tavole rotonde hanno animato il dibattito. La prima, moderata dal prof. Saverio Minucci, Direttore di un'unità di ricerca di IEO, ha esplorato le sinergie tra industria e istituti di ricerca per favorire l'innovazione clinica. Tra i partecipanti il prof. Giuseppe Curigliano, Direttore della Divisione di Sviluppo di Nuovi Farmaci per Terapie Innovative, Ing. Annarosa

Farina, Direttore dell'Area Sistemi Informativi di IEO, prof. Nicola Fusco, Direttore della Divisione di Anatomia Patologica di IEO, dott. Holger Neecke, Amministratore Delegato di Tethis SpA e dott.ssa Marcella Origgi, Early Innovation Partner – Italy presso Johnson & Johnson.
La seconda tavola rotonda, guidata dalla dott.ssa Marzia Fumagalli, si è focalizzata sul tema dell'imprenditorialità e della creazione di start-up nelle scienze della vita, con interventi di dott. Fabio Bianco, Direttore Scientifico di Bio4Dreams, prof. Roberto Chiarle, Direttore della Divisione di Diagnosi Emolinfopatologica di IEO, dott.ssa Federica Draghi, Fondatore e Partner Responsabile di XGEN Venture SGR, prof. Saverio Minucci e dott. Alessandro Tozzi, Socio Fondatore e Amministratore Delegato di Endostart Srl. Il prof. Ennio Tasciotti, Direttore del Human Longevity Program dell'IRCCS San Raffaele di Roma, ha evidenziato come gli errori possano rappresentare un'importante fonte di ispirazione per l'innovazione, suscitando spunti di riflessione stimolanti tra i partecipanti. La giornata si è conclusa con una sessione, moderata dalla dott.ssa Claudia Iavarone, Senior Specialist presso IEO-TT, di presentazioni di business pitch di alcuni progetti innovativi sviluppati in IEO. Tra i relatori: prof. Salvatore Pece, dott.ssa Lucilla Titta, dott. Gioacchino Natoli, prof. Pier Paolo Di Fiore e il dott. Luigi Nezi.

News, initiatives and events from the IEO world!

Urologia d'eccellenza: IEO riceve il riconoscimento del Bollino Azzurro da Fondazione Onda ETS.

IEO ha ricevuto da Fondazione Onda ETS il riconoscimento del Bollino Azzurro, che valorizza l'eccellenza nella prevenzione, diagnosi, cura e riabilitazione in ambito uro-andrologico. Questo traguardo è il risultato del lavoro e della dedizione di tutto il personale IEO, unito dall'obiettivo di migliorare la qualità della vita dei pazienti e promuovere una maggiore consapevolezza sulla salute maschile.

News, initiatives and events from the IEO world!

Christmas Gala per la Ricerca – 30th Anniversary: raccolti 415.000 euro a favore della Fondazione IEO-MONZINO ETS.

La sera del 28 novembre si è tenuto il tradizionale Christmas Gala per la Ricerca della Fondazione IEO-MONZINO ETS, il charity dinner dedicato a donatori, medici, ricercatori e sostenitori fedeli, riuniti in una serata speciale, organizzata nei nuovissimi spazi Rubattino Studio | R56 di Milano.
Quest'anno il Gala ha avuto un significato particolare, celebrando i 30 anni dello IEO e della Fondazione nata nel 1994 per sostenere la Ricerca dello IEO e del Monzino.
Sotto la guida di Patrizia Sandretto Re Rebaudengo, la Fondazione ha esteso negli anni il suo raggio d'azione, affiancando all'impegno clinico-scientifico attività di sensibilizzazione per diffondere la cultura della prevenzione e promuovere il valore della ricerca oncologica e cardiovascolare.
La serata è stata resa possibile grazie al prezioso sostegno di numerosi partner e alla collaborazione di Ploom PR di Carla e Alessandro Cordiano, agenzia specializzata in comunicazione globale ed eventi. A rendere unico il Christmas Gala è stata la generosità dei 700 ospiti che, partecipando alla cena e all'asta benefica silenziosa, hanno contribuito a raggiungere un risultato straordinario per la ricerca medica, 415.000 euro.
La Fondazione esprime la sua profonda gratitudine a tutti coloro che hanno reso possibile l'evento, dalla generosità degli ospiti alle aziende sponsor – Allianz, Gilead Kite, LabAurelia Group, MIAMO, Pellegrini e REPLY -, dallo staff organizzativo agli artisti che hanno donato il loro tempo e talento per una causa nobile. La serata è stata un successo dell'impegno per il bene comune.

IEO MEMBERS - LET'S GET TO KNOW THEM BETTER.

Elena Dal Zotto, grant officer.

Elena - La Signora Wolf dei Grant (con un debole per la parmigiana di melanzane).

Sono una Grants Officer con una missione: trasformare le idee brillanti di ricercatori e medici in progetti finanziati. Sono l'arma segreta che permette loro di concentrarsi sulla scienza, mentre io districo bandi, budget e rendiconti. Il Signor Wolf di Pulp Fiction, ma con i fogli di calcolo.

In 4 anni in IEO ho affinato le mie capacità di progettazione e gestione di progetti europei, dribblando scadenze serrate, bandi folli e audit implacabili. Partecipo al network EU-LIFE coordinando il gruppo di lavoro Grants & Funding Strategies. Ai tavoli europei trovo la mia dimensione, perché mi permettono di sapere dove tira il vento delle politiche di finanziamento alla ricerca e di riportare le esigenze dei ricercatori con cui lavoro.

Non ho il camice bianco, ma ho una mente analitica, una grande fiducia nel rigore scientifico (anche se leggo l'oroscopo) e una passione per gli ambienti creativi che pongono sfide intellettuali. Sono laureata in Economia Politica e dello Sviluppo. La scelta di concentrarmi sulle economie emergenti riflette il mio interesse per i contesti internazionali con un futuro ancora tutto da scrivere.

Cintura nera di judo, ho fatto mia una disciplina che insegna l'importanza del miglioramento continuo e di un impiego dell'energia bilanciato e indirizzato. Nel tempo libero mi ricarico praticando yoga e organizzando cammini. Amo viaggiare, perché con lo zaino in spalla esprimo la mia versione migliore, quella più aperta alla scoperta e al cambiamento.

Il mio obiettivo è continuare a supportare la ricerca scientifica, aiutando i ricercatori ad avanzare nella conoscenza dell'oncologia e a migliorare la qualità della vita delle persone, un grant alla volta.

What's new in science?

Disrupting circulating tumor cell aggregates to reduce cancer cell metastatic potential by exploiting a drug for heart conditions.

This first in-human study showed that the administration of digoxin, a drug commonly used for the treatment of mild-to-moderate heart conditions, in metastatic breast cancer patients partially disrupts aggregates of circulating tumor cells in the blood, reducing their metastatic potential, likely affecting patient clinical outcome.

The metastatic disease represents the main cause for breast cancer-related death; yet, specific metastases-targeted drugs are not currently available.

Metastases arise when tumor cells shed from the primary tumor, invade the surrounding tissue, move into the blood vessels and, through the bloodstream, reach distant organs where they seed new tumors: The metastases. In the bloodstream, circulating tumor cells (CTCs) "travel" mostly aggregated in groups (the so-called CTC clusters). CTC clusters have higher probability of seeding metastases upon reaching the distant organ as compared to single CTCs. In breast cancer patients (and other tumor types), the presence of CTCs in the bloodstream has been associated with distant metastases and poor patient survival. Previous studies in preclinical in vivo models showed that administration of drugs dissolving CTC aggregates, such as the NA/K ATPase inhibitor digoxin, suppress metastases.

Main finding. A recent paper reports the results of the first in-human study assessing, in metastatic breast patients, the disrupting effect on CTC clusters of the administration of digoxin, a drug commonly used for the treatment of mild-to-moderate heart conditions.

Details. The authors assessed the effect of the administration, in breast cancer patients, of digoxin, at safe and well tolerated doses (0.125-0.250 mg per day), on the dissolution of CTC clusters and on the number of CTC clusters in the blood, as well as the kinetics of cluster dissolution and the dose-response relation.

CTCs isolated from blood samples of digoxin-treated breast cancer patients and control non-digoxin-treated breast cancer patients were analyzed, by considering size, number and "type" of CTC clusters (that is, containing cancer cells only (homotypic) or both cancer cells and immune cells (heterotypic)). Firstly, while digoxin-treated CTC clusters displayed reduced size, no CTC cluster size reduction was observed in non-digoxin-treated control patients. Moreover, by comparing cluster size before and after digoxin treatment, they found a significant cluster size reduction after digoxin. Higher digoxin concentration in the blood

Digoxin

Digoxin is an FDA-approved drug for the treatment of mild-to-moderate heart conditions. Although, after its approval in 1954, its use has been largely replaced (for rate control) by beta blockers and calcium channels blockers, it is still typically employed when other drugs are ineffective, basically resulting in slowing down the heart rate. Usually administered intravenously, less common are the intramuscular injections. Known risks of toxicity are associated with fatal cardiac arrhythmias; namely, irregular heartbeats, (estimated incidence is around 0.8% to 4% of patients on steady digoxin therapy) and increase with increasing blood concentrations of the drug (>2ng/mL).

appeared also correlated with reduced cluster size, even though the correlation was not statistically significant. Importantly, no adverse events related to digoxin administration were observed.
In preclinical in vivo models, they observed a correlation between digoxin-elicited reduced cluster size and disease outcome, with larger CTC clusters endowed with greater metastatic potential (that is, they found CTC metastatic ability being a function of CTC cluster size).
Finally, transcriptomic profiling of patient-derived CTCs at different timepoints, before and after digoxin (3 samples collected before digoxin administration and 1 samples collected after digoxin) unveiled the differential expression level of 708 genes (the majority of these genes being downregulated after treatment). Differentially expressed genes were involved in cell cycle and cell-cell adhesion, confirming previous data describing the loss of cell-cell adhesion and the interference with cell cycle following inhibition of the Na+/K+ ATPase.
Conclusions. Previous studies have shown that clusters of CTCs in the blood are associated with metastases and poor patient prognosis. In this work, the authors found that in metastatic breast patients, with progressive disease which recurred after previous treatment, and for whom no other curative therapy option is currently available, the administration of digoxin, at well tolerated doses, decreases the size of CTC clusters in the blood, through the reduced expression of genes involved in cell-cell adhesion, thus likely affecting metastasis colonization and patient prognosis.
From a clinical point of view, despite the significant -yet mild- effect of digoxin on CTC cluster size, and the lack of direct assessment of clinical outcome in patients (although they demonstrated in preclinical in vivo models the reduced metastasization after digoxin-induced CTC cluster dissolution), these results suggest that further investigation of approaches aimed at interfering with CTC metastatic potential, through the interference with CTC cluster size, may be a viable approach for preventing metastatic colonization. Indeed, the scientific literature indicating the correlation between CTC clusters and patient prognosis suggests that digoxin may interfere with patient prognosis. However, other factors might need to be considered, such as the effect of interfering with cell-cell adhesion on the primary tumor. Anyway, from a biological point of view, this work represents a proof of concept, showing the feasibility of the pharmacological CTC cluster disruption through the inhibition of the NA/K ATPase, likely exploitable to reduce the metastatic potential of these cancer cells through the repurposing of a drug for heart conditions. The authors suggest also the usefulness of future studies aimed at testing more potent or selective drugs, which may have more evident effects, higher dosages or prolonged treatments, in order to design novel, effective and refined approaches.

Reference. Digoxin for reduction of circulating tumor cell cluster size in metastatic breast cancer: a proof-of-concept trial. *Christian Kurzeder, Bich Doan Nguyen-Sträuli, Ilona Krol, Alexander Ring, Francesc Castro-Giner, Manuel Nüesch, Simran Asawa, Yu Wei Zhang, Selina Budinjas, Ana Gvozdenovic, Maren Vogel, Angela Kohler, Cvetka Grašič Kuhar, Fabienne D. Schwab, Viola Heinzelmann-Schwarz, Walter Paul Weber, Christoph Rochlitz, Denise Vorburger, Heike Frauchiger-Heuer, Isabell Witzel, Andreas Wicki, Gabriela M. Kuster, Marcus Vetter, Nicola Aceto*. Nature medicine 2025. doi: 10.1038/s41591-024-03486-6.

What's new in science?

Personalized vaccines against renal cancer: Results of a phase I clinical trial.

A phase I clinical trial evaluated safety, effect on the immune system activity and efficacy of a personalized tumor vaccine in patients with renal cancer, who underwent surgery for primary tumor resection and are at high risk of disease recurrence. Results showed that the vaccine was overall well-tolerated, effective in controlling relapse up to three years after surgery and able to stimulate an antitumor immune response.

When mutations in a cancer cell lead to the generation of abnormal proteins, not present in normal cells, these can be recognized by the immune system. These proteins are called neoantigens and can stimulate an antitumor immune response specifically against the tumor cell expressing that protein/neoantigen. Neoantigens can be exploited to produce personalized cancer vaccines, able to stimulate a neoantigens-guided, specific immune response, more effective and less toxic.

Main finding. In a recent paper, the authors reported the results of a phase I clinical trial investigating safety, effects on immune system activity, and antitumor efficacy of a peptide-based, personalized, neoantigen-targeting cancer vaccine in renal cancer patients who underwent complete surgical resection of the primary tumor, at high risk of recurrence. The results showed that the vaccine was overall well-tolerated, efficiently controlled tumor recurrence up to two years after surgery and able to stimulate an antitumor immune response.

Details. Nine patients in advanced stage renal cancer were enrolled in the study. Tumor samples (and, in the two patients who had metastases, metastatic samples too) were sequenced to identify mutations predicted to generate neoantigens. From these mutated sequences, short fragments of protein were generated, pooled (in four different pools; each pool administered to the patient contained five different peptides) and administered to the patients, either alone or in combination with immunotherapy (ipilimumab).

Safety and clinical efficacy. No severe adverse events were observed (only inflammatory skin reactions at the injection site and transient flu-like symptoms were noted) and none of the nine patients enrolled experienced recurrence (at a median followup of 40.2 months after surgery). No differences were observed in patients (intradermally and subcutaneously) injected with the vaccine only and those administered the vaccine along with ipilimumab.

Immune system reactivity. How did the immune system react? T cells were collected from patients at different time points after vaccination and their reactivity, ex vivo, against peptides of the pool as well as patient-derived tumor cells was analyzed. Although the timing of reactivity varied, T cells did react upon stimulation. Once again, no differences were observed in patients receiving the vaccine only as compared to those receiving also ipilimumab. Notably, the most common driver mutations in this cancer type showed high immunogenicity (high capability of inducing immune response) when tested in vitro. Comparative analyses, before *vs* after vaccination, of the inflammatory skin reaction at the injection site revealed high infiltration of immune (myeloid and lymphoid) cells.

Furthermore, analysis of the blood of vaccinated patients revealed an increased concentration of cytokines involved in T cell activity and cytotoxicity. Importantly, factors involved in suppressing the immune response also increased, indicating a coordinated remodelling of immune responses following vaccination. Finally, immune response to vaccination was durable: Up to three

"these results suggest the potential of this approach in the adjuvant setting, for instance in the presence of micrometastases, after surgery, to clear potential clinically undetectable residual disease"

years after injection, vaccine-induced expanded patients' T cells were able to recognize, in vitro, the patients' tumors (overall, antitumor reactivity was detected in 77.8% of patients.)

Conclusions. Although the number of patients involved in the study is limited, the results of this trial are promising. The authors showed the clinical efficacy of this approach based on the sequencing of patients' tumor tissues for the generation of personalized, peptides-based vaccines able to stimulate the patients' own immune system against the tumor, and prevent tumor recurrence in high risk patients. Notably, the study was conducted in patients with tumors known to be characterized for having a low mutational burden, that is low number of mutations and thus less likely to produce potential neoantigens to stimulate the immune system. These results suggest the potential of this approach in the adjuvant setting, for instance in the presence of micrometastases, after surgery, to clear potential clinically undetectable residual disease, perhaps in combination with immunotherapeutics (other than ipilimumab, which showed poor efficacy) to potentiate the immune response.

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What's new from IEO Researchers?

TBC1D proteins between cell metabolism and cancer cell aggressiveness.

Cancer cells are endowed with a "metabolic plasticity", namely the capability to adapt to the higher energy demands that correlate with the tumorigenic status by reprogramming their metabolism. TBC1D proteins are critical regulators of intracellular trafficking and impact, through this regulation, on the cellular metabolic state. In a recent paper by Lupi, Avanzato, Confalonieri et al., the authors, co-directed by Pier Paolo Di Fiore -Group Leader at the department of experimental oncology of IEO and professor at the University of Milan- and Letizia Lanzetti (Candiolo Cancer institute), found that TBC1D proteins -and in particular TBC1D7- are involved in the reprogramming of cell metabolism that correlates with triple-negative breast cancer (TNBC) patient prognosis: Namely, a high level of expression of TBC1D7 protein in cancer cells alters the intracellular trafficking of GLUT1 protein, in turn modifying its abundance at the cell membrane and sustaining the deep change of cancer cell metabolism (towards the glycolytic pathway) featuring an aggressive disease.

Pier Paolo Di Fiore and Letizia Lanzetti

Although TNBC is overall an aggressive tumor, it is clinically rather heterogeneous and some TNBC patients have a better prognosis. In this scenario, firstly, TBC1D7 represents a promising candidate for the clinical management of patients, identifying those with a better prognosis who may avoid useless aggressive treatments. Secondly, the correlation between TBC1D7 and cell metabolism makes it an interesting potential pharmacological target to interfere with alterations of cell metabolism that characterize cancer cells.

TELL ME MORE!

By exploiting the METABRIC dataset, the authors found that the expression of TBC1D proteins (TBC domain-containing proteins, TBC1Ds) was often altered in TNBC as compared to other breast cancer molecular subtypes and it actually mirrored patient prognosis; in particular, high levels of TBC1Ds correlated with worse patient prognosis.

Furthermore, the expression of eleven TBC1D genes was associated with an altered metabolic profile in breast cancer cells. One group of TBC1D genes correlated with high levels of metabolites of the glycolytic pathway and with genes typically overexpressed in TNBC, whereas another group was associated with high levels of metabolites of fatty acid oxidation pathway and genes commonly underexpressed in TNBC. Further analyses revealed a causal relation between TBC1Ds expression and the glycolytic pathway: TBC1Ds silencing downregulated the glycolytic pathway and reduced mitochondrial activity, indicating a reprogramming of cancer cell metabolism towards a less energetic state following TBC1D silencing.

By specifically focusing on TBC1D7, the authors showed that TBC1D7-mediated modulation of cell metabolism was exerted, partly, through regulation of membrane trafficking and the resultant change of GLUT1 abundance on the cell surface and, partly, through a transcriptional regulation, although how TBC1D7 transcriptionally regulates cell reprogramming is

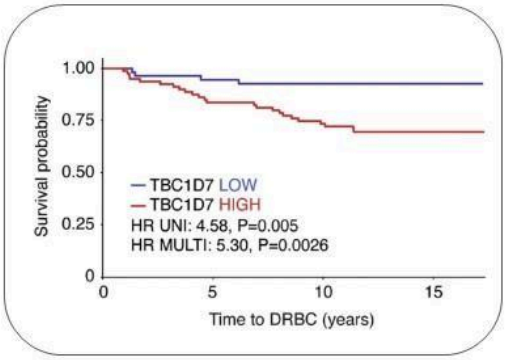

Figure adapted from Lupi et al., an open access article under the CC-BY licence

unclear. Indeed, by exploiting a mutant TBC1D7 unable to bind the (TSC1/TSC2) protein complex regulating mTORC1 activity, the authors showed that there was no correlation with mTORC1, whose pathway is known to be repressed by TBC1D7.

Furthermore using a large cohort of IEO patient samples, the authors confirmed that, at the protein level as much as at the RNA level, TBC1D7 distinguished TNBC patients in two groups according to their prognosis. Moreover, TBC1D7 silencing affected the glycolytic pathway only in those cells that (before silencing) had high levels of TBC1D7, while it basically did not have any effect in those cells that (before silencing) had low levels of TBC1D7, indicating that in these cells, high levels of TBC1D7 expression are needed to maintain active glycolysis. Consistently, cell metabolism genes -in particular, many genes of the glycolytic pathway- were downregulated in TBC1D7-silenced cells.

Reference. TBC1 domain-containing proteins are frequently involved in triple-negative breast cancers in connection with the induction of a glycolytic phenotype. *Mariadomenica Lupi #, Daniele Avanzato #, Stefano Confalonieri #, Flavia Martino, Rosa Pennisi, Emanuela Pupo, Valentina Audrito, Stefano Freddi, Giovanni Bertalot, Francesca Montani, Bronislava Matoskova, Sara Sigismund, Pier Paolo Di Fiore*, Letizia Lanzetti**. Cell Death Dis 2024. doi: 10.1038/s41419-024-07037-2.

What's new from IEO Researchers?

From preclinical studies, a novel biomarker and pharmacological target in bladder cancer.

Pece Lab

Although the majority (75%) of bladder cancers are non-invasive and have overall a good prognosis, these tumors often relapse and progress towards an infiltrating, invasive disease which has a poor prognosis, despite the currently available therapeutic options, associated with severe side effects and poor quality of life. A challenge in the optimal treatment of bladder cancer patients is the lack of biomarkers, preventing the estimate of the risk of the non-invasive disease to progress into an invasive disease, and thus distinguishing patients who may simply undergo active surveillance from those that are at high risk and need more aggressive treatments. Another critical aspect is the scarcity of effective targeted therapies.
In a recent paper by Tucci, Pennisi et al., funded by AIRC, the authors co-directed by Salvatore Pece and Daniela Tosoni –PI and researcher at the department of experimental oncology of IEO, respectively– found that NUMB protein is a prognostic and predictive marker of bladder cancer progression, as its loss is associated with high risk of disease progression from invasive to non-invasive disease. Moreover, NUMB plays an active role in tumorigenesis, as its loss is sufficient to induce tumor onset, and, in already transformed bladder cancer cells, it enhances aggressiveness, conferring migratory and infiltrating features. They dissected the underlying molecular mechanisms and proposed NUMB –and the related RhoA-Rock-YAP pathway– as a novel therapeutic target to fight cancer progression.

By identifying NUMB as a novel biomarker of disease progression/aggressiveness, this study contributes to improving the current clinical management of bladder cancer patients. Indeed, NUMB presence/absence –as well as the 27-gene signature associated with NUMB loss– could be exploited in the clinical setting, along with currently available clinicopathological parameters as a marker to distinguish high risk from low risk patients. Moreover, due to its active role in tumorigenesis, NUMB represents also a potential novel pharmacological target to arrest bladder cancer progression. Notably, several drugs that interfere with the identified pathways associated with NUMB loss are already used in a clinical setting, such as the Verteporfin (YAP inhibitor), employed in ophthalmology conditions, or fasudil (ROCK inhibitor), in clinical trial for vascular and neurodegenerative diseases, indicating the possibility of a rapid drug repurposing in bladder cancer treatment.

“Our discovery –comments Salvatore Pece, PI at IEO and full professor of the university of Milan– has a strong and immediate potential application in the clinical practice, demonstrating that superficial and deep bladder tumors represent different stages of the same pathological process, that evolves over time, driven from the outset by specific molecular mechanisms that can be targeted with drugs. It becomes crucial, therefore, to identify the biological mechanisms underlying this evolution and develop new molecular markers to identify patients with specific aggressive characteristics.”
“We have shown that it is possible to inhibit the proliferative and invasive capacity of NUMB-deficient tumor cells –continues Daniela Tosoni, researcher of IEO and of the university of Milan– by using drugs that can target this complex molecular circuit at multiple levels. Bladder tumors lacking NUMB are indeed very aggressive, but they are also highly vulnerable.”
“We also identified –adds Salvatore Pece– a new molecular signature that will allow for the accurate identification of patients who could benefit from targeted treatments with new drugs that specifically target the molecular mechanisms activated by the loss of NUMB.”
“The study, which saw scientists and clinicians from our institute working together in a common effort –concludes Roberto Orecchia, Scientific Director of IEO– is an extraordinary result. We have already patented the new molecular signature that emerged from this research and are about to launch clinical studies to validate its use as a marker to identify patients at high risk of disease progression, who will be able to benefit in the near future from a new therapeutic perspective with more precise and targeted drugs.”
Read the press release here (Italian only).

TELL ME MORE!

IEO researchers previously showed a role of NUMB protein in breast cancer tumorigenesis (Filippone et al., J Cell Biol 2022). In this work, the authors found that NUMB expression was frequently downregulated in bladder cancer samples as compared to the healthy tissue and its expression levels correlated with disease aggressiveness and mortality: Low NUMB expression in non-invasive tumors was predictive of the progression into an aggressive, invasive disease, independently from other factors (such as sex, age, TNM stage), showing its potential in patient stratification into risk classes.
The transcriptional profiling of both male and female, NUMB-low and NUMB-high bladder cancer cell lines enabled the identification of a 27-gene signature predictive of high risk of disease progression, distinguishing NUMB-low patients, having higher risk of progression into an invasive disease, from NUMB-high patients.
Detailed analysis of the underlying molecular mechanisms revealed the cell pathways implicated: The reduced NUMB expression led to the hyper-activation of the RHOA/ROCK pathway, which in turn impacted on the actin cytoskeleton. That resulted in the inhibition of the Hippo pathway, with the ensuing activation of the YAP pathway and the epithelial-to-mesenchymal transition (EMT) program, most likely responsible for the acquisition of the more aggressive/invasive cell behavior of NUMB-low bladder cancer cells. The targeted drug inhibition, at different stages, of the molecular cascade downstream of NUMB loss significantly limited cancer cell invasion; these drugs selectively hit NUMB-lacking tumor cells,

being at the same time ineffective on NUMB-expressing cells.
NUMB-deficient bladder cancer cells also displayed a different morphology as compared to NUMB-proficient cells: They displayed reduced roundness, irregular shape, with protrusions, increased cell area and loss of cell-cell adhesion, indicating an invasive phenotype. Notably, pharmacological inhibition of the NUMB-related cell pathways and ectopic re-expression of NUMB partially reversed the invasive phenotype. Interestingly, NUMB loss led to the appearance of infiltrating neoplastic lesions, and increased aggressiveness of already transformed cells, accelerating the progression from non-invasive to invasive tumors.
Findings in *in vitro* and *in vivo* mouse models were confirmed in human-derived preclinical models, highlighting the clinical relevance of these findings.
Therefore, by combining murine and human-derived *in vitro* cultures, as well as *in vivo* murine preclinical models and patient data, pharmacological inhibition and gene silencing, this study shows that, in line with previous studies demonstrating a role of NUMB in breast cancer tumorigenesis, NUMB acts as a tumor suppressor in bladder cancer. Indeed, on one side, its loss accelerates disease progression from non-invasive to invasive disease; on the other side, it is sufficient alone to induce tumorigenesis. NUMB represents at the same time a biomarker of disease aggressiveness and an actionable target, in bladder cancer and, perhaps, in other tumor types.

Reference. Loss of NUMB drives aggressive bladder cancer via a RHOA/ROCK/YAP signaling axis. *Tucci FA, Pennisi R, Rigiracciolo DC, Filippone MG, Bonfanti R, Romeo F, Freddi S, Guerrera E, Soriani C, Rodighiero S, Gunby RH, Jodice G, Sanguedolce F, Renne G, Fusco N, Di Fiore PP, Pruneri G, Bertalot G, Musi G, Vago G, Tosoni D, Pece S.* Nat Commun. 2024. doi: 10.1038/s41467-024-54246-6.

How do cells cope with aneuploidy? The characterization of the underlying molecular mechanisms reveals therapeutically exploitable cancer cell vulnerabilities.

Stefano Santaguida and Marica Ippolito.

An abnormal number of chromosomes –aneuploidy– is a common feature of cancer cells, induces genomic instability, DNA damage and cell stress. Aneuploid cells must find a way to manage such abnormalities in order to survive and proliferate. An in-depth, molecular-level understanding of how cells cope with aneuploidy-related stress may reveal targetable vulnerabilities of aneuploid *cancer* cells potentially exploitable with therapeutic scopes to selectively kill stressed aneuploid cancer cells.

In two recent papers by Ippolito, Zerbib et al. published in *Cancer Discovery* and *Nature Communications*, the authors co-supervised by Stefano Santaguida –Group Leader at the Department of Experimental Oncology of IEO and professor at the University of Milan– and Uri Ben-David –of the University of Tel Aviv– performed a genomic, transcriptomic and proteomic profiling of aneuploid cells, showing that in order to survive and proliferate, aneuploid cells –both untransformed and malignant– evolved specific mechanisms to cope with aneuploidy-related stress.

In particular, the authors demonstrated that, on one side, aneuploid cells activate mechanisms to degrade the excess of RNA and protein synthesized, which involve the activation of the proteasome; on the other

side, through the increased activity of the RAF/MEK/ERK pathway, aneuploid cells overcome the high DNA damage level characterizing aneuploidy, or occurring for instance during chemotherapy causing chemoresistance.

The identification of these survival mechanisms in aneuploid cells has relevant therapeutic implications. Indeed, on one side, aneuploidy makes these cells sensitive to inhibitors of the RAF/MEK/ERK pathway and pharmacological inhibition of RAF/MEK/ERK pathway in turn renders aneuploid cells sensitive to DNA damage-inducing drugs –such as chemotherapeutics– thus paving the way to new and effective drug combinations. On the other side, the need to activate such mechanisms to buffer the extra RNA and protein associated with an additional chromosome to survive and proliferate makes these cells vulnerable and the pharmacological inhibition of the mechanisms degrading the excess of RNA and proteins kills aneuploid cells. Such vulnerabilities of aneuploid cells could thus be exploited in a clinical setting; for instance, some inhibitors of the proteasome –such as bortezomib– are clinically approved and employed for patient treatment. Moreover, these results indicate that (the degree of) aneuploidy can represent a marker to identify potential responders to proteasome inhibitor-based therapy.

TELL ME MORE!

First, the authors developed a proper system to explore aneuploidy in untransformed cells, offering at the same time to the scientific community a useful tool for aneuploidy studies. Through chemical treatment, they randomly induced aneuploidy in RPE cells; then, they selected six cell populations with different, increasing degrees of aneuploidy: Low level of aneuploidy (LLA) cells and high level aneuploidy (HLA) cells (with complex karyotype).

The molecular (genomic, transcriptomic and proteomic) characterization of these cells enabled the identification of a transcriptional signature related to the overall aneuploidy state rather than to the specific chromosome gained or lost. The aneuploidy signature was characterized by the upregulation of genes of the DNA damage response and repair (DDR), as well as by the upregulation of RNA metabolism pathways and proteotoxic stress (as well as by the downregulation of cell proliferation-related genes), suggesting that aneuploid cells were able to cope with high levels of DNA damage (DD). The characterization of these aneuploid cells in terms of drug sensitivity revealed that although HLA cells showed higher level of DD, due to the up-regulation of DDR genes, they were also more tolerant and thus more resistant to DD-inducing drugs, such as topotecan and etoposide –two chemotherapeutics commonly used in a clinical setting– and olaparib – a clinically approved PARP inhibitor. These results were confirmed in other models of aneuploid cells, both untransformed and malignant. Despite the overall greater drug resistance of aneuploid cells to DD-inducing drugs, which could survive and proliferate due to the upregulation of DDR genes, these cells displayed other vulnerabilities, such as higher sensitivity to inhibitors of the RAF/MEK/ERK pathway (which was particularly evident in HLA cells) and, specifically, to c-RAF inhibition. Interestingly, c-RAF was activated in HLA but not in LLA cells. c-RAF activation was an early event after the induction of aneuploidy, suggesting that it was an adaptive mechanism, both in untransformed and cancer aneuploid cells, needed to overcome DD. c-RAF is usually activated by DDR and, indeed, c-RAF inhibitor in combination with DD-inducing drugs killed aneuploid cells: c-RAF inhibition rendered aneuploid cells sensitive to DD-inducing drugs such as etoposide and olaparib. Moreover, both untransformed and cancer aneuploid cells were sensitive to clinically approved MEK inhibitors such as trametinib (and selumetinib), and the ERK inhibitor ulixertinib. MEK/ERK pathway hyperactivation thus represented the mechanism of resistance of aneuploid cells, and the inhibition of this pathway sensitized cells to DD-inducing drugs –such as chemotherapeutics–, supporting the antitumor efficacy of a combination therapy with inhibitors of DDR and MEK/ERK pathway. Finally, by analyzing datasets of pancreatic and breast cancer patients, they observed that, in these patients, resistance to olaparib reflected the hyperactivation of MEK/ERK pathway, which was specific to aneuploid tumors (Zerbib, Ippolito et al., Nature communications 2024).

The molecular characterization of aneuploid cells also highlighted the upregulation of genes associated with RNA and protein modulation, both in untransformed and tumor aneuploid cells; specifically, genes related to RNA metabolism and gene silencing as well as genes of

the unfolded protein response (UPR) pathway and protein degradation, indicating that aneuploid cells deployed these mechanisms to attenuate the excess of RNA and proteins associated with aneuploidy. Notably, in agreement with previous studies (Senger et al., Elife 2022), the buffering mechanism was particularly relevant for proteins that were part of multiprotein complexes. In particular, regarding RNA levels, not only did aneuploid cells show higher RNA synthesis rates, but they also displayed greater RNA degradation due to the overexpression of the NMD (nonsense-mediated decay) pathway, induced by the aneuploidy-elicited DDR (DNA damage response) activation. NMD-mediated RNA degradation enabled aneuploid cell proliferation; therefore, aneuploid cells were more dependent on this pathway and thus more sensitive to NMD inhibiting drugs. Aneuploidy was associated with the overexpression of NMD genes also in human tumors. In addition to NMD-mediated RNA degradation, mechanisms of gene expression silencing via miRNAs also played a key role in buffering the excess of RNA. Therefore, aneuploid cells deal with the stress associated with RNA in excess by deploying NMD- and miRNA- mediated mechanisms of RNA degradation, which, while enabling aneuploid cells to survive, at the same time represent a vulnerability, to be targeted in order to kill them. Similarly, aneuploidy-related proteins in excess induced proteotoxic stress, leading to the upregulation of the UPR -the primary response of aneuploid cells to proteotoxic stress-, resulting in the attenuation of protein translation. Furthermore, these cells displayed a greater activation of proteasome-mediated protein degradation, reliance of aneuploid cells on proteasome activity and, as a consequence, sensitivity of aneuploid cells to proteasome inhibition. Aneuploid cells upregulated proteasome activity in response to proteotoxic stress and that made them more sensitive to proteasome inhibition: bortezomib -a clinically approved proteasome inhibitor- killed aneuploid cells. Consistently, gene expression analysis of cancer patients data revealed that the majority of patients treated with bortezomib and manifesting complete response had tumors with greater aneuploid degree as compared to those that showed a progressive disease, highlighting that response to the proteasome inhibitor bortezomib correlated with aneuploidy degree and indicating that the degree of aneuploidy can be used as a marker predicting response to proteasome inhibitor therapy in cancer patients, underlining the clinical relevance of these findings (Ippolito, Zerbib et al., Cancer Discovery 2024).

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Increased RNA and Protein Degradation Is Required for Counteracting Transcriptional Burden and Proteotoxic Stress in Human Aneuploid Cells. *Marica Rosaria Ippolito #, Johanna Zerbib #, Yonatan Eliezer, Eli Reuveni, Sonia Viganò, Giuseppina De Feudis, Eldad D Shulman, Anouk Savir Kadmon, Rachel Slutsky, Tiangen Chang, Emma M Campagnolo, Silvia Taglietti, Simone Scorzoni, Sara Gianotti, Sara Martin, Julia Muenzner, Michael Mülleder, Nir Rozenblum, Carmela Rubolino, Tal Ben-Yishay, Kathrin Laue, Yael Cohen-Sharir, Ilaria Vigorito, Francesco Nicassio, Eytan Ruppin, Markus Ralser, Francisca Vazquez, Stefano Santaguida*, Uri Ben-David**. Cancer Discov 2024. doi: 10.1158/2159-8290.CD-23-0309.

Human aneuploid cells depend on the RAF/MEK/ERK pathway for overcoming increased DNA damage. *Johanna Zerbib #, Marica Rosaria Ippolito #, Yonatan Eliezer, Giuseppina De Feudis, Eli Reuveni, Anouk Savir Kadmon, Sara Martin, Sonia Viganò, Gil Leor, James Berstler, Julia Muenzner, Michael Mülleder, Emma M Campagnolo, Eldad D Shulman, Tiangen Chang, Carmela Rubolino, Kathrin Laue, Yael Cohen-Sharir, Simone Scorzoni, Silvia Taglietti, Alice Ratti, Chani Stossel, Talia Golan, Francesco Nicassio, Eytan Ruppin, Markus Ralser, Francisca Vazquez, Uri Ben-David*, Stefano Santaguida**. Nat Commun 2024. doi: 10.1038/s41467-024-52176-x.

What's new from IEO Researchers?

Targeted treatment of HER2-negative breast cancer: Results of a phase III clinical trial.

HER2 (Human epidermal growth factor 2)-negative breast cancer (BC) is the most common BC subtype. These tumors are quite heterogeneous in terms of HER2 expression levels and, on the basis of HER2 expression, (measured trough immunohistochemistry or in situ hybrdization) they can be classified in either HER2-low and HER2-ultralow. The current standard of care for HER2-negative metastatic BC patients is first line treatment with endocrine therapy plus CDK4/6 inhibitors: however, in case of disease progression the optimal treatment approach is not well defined.

Giuseppe Curigliano

In a paper recently published in the New England Journal of Medicine, in the frame of an international collaboration coordinated by Giuseppe Curigliano -IEO Scientific Deputy Director and Director of the IEO Division of New Drugs Development for Innovative Therapies- in the context of the phase III DESTINY-Breast06 trial, the authors evaluated efficacy and safety of the HER2-targeted antibody-drug conjugate trastuzumab deruxtecan (TDX) as compared to chemotherapy in hormone receptor-positive metasttic BC patient with very low levels of HER2.

TDX displayed significant benefits in both HER2-low and HER2-ultralow metastatic BC patients, with longer progression-free survival as compared to the standard chemotherapy approach. Interestingly, TDX proved to be more effective independently from HER2 expression levels (as it was effective in the whole patient population, either HER2-low or HER2-ultralow, possibly due to HER2 presence -though at low levels- on cell membrane), or prior treatment with CDK4/6 inhibitors, or the specific chemotherapeutic they were compared to.

Notably, based on the results of the previous DESTINY-Breast04 trial, TDX was already approved for the treatment of metastatic patients who progressed on previous chemotherapy. However, in the frame of this DESTINY-Breast06 study, the authors proved efficacy of TDX also in other patient groups at earlier treatment stages, namely those that have not yet received chemotherapy.

TELL ME MORE!

The DESTINY-Breast06 is a multicenter phase III trial, enrolling 866 patients (713 HER2-low and 153 HER2-ultralow), across 324 sites, with metastatic disease who progressed after standard treatment. Enrolled patients were randomly assigned to receive either TDX (436 patients) or chemotherapy (430 patients). The authors evaluated both safety and efficacy of the treatment, in terms firstly of progression free survival (PFS) and secondly overall survival (OS), as well as objective response and duration of response. The analyses were conducted in on the whole patient population enrolled, namely including the HER2-ultralow (with HER2 staining score = 0) and HER2-low (with HER2 staining score = 1 or 2) as well as in the HER2-low subgroup separately.

Efficacy. TDX treatment was more effective than chemotherapy, with a significantly longer PFS in TDX-treated patients as compared to those receiving chemotherapy, both in the HER2-low and in the whole population, (namely, including both HER2-low and HER2-ultralow. Results on OS collected so far were not mature; moreover, as in these patients survival after disease progression is longer, subsequent anticancer therapies are likely to have a significant effect on OS which should be taken into consideration. Results showed greater efficacy of TDX also considering duration of response and objective response; some TDX-treated patients displayed complete response (while no chemotherapy-treated patients showed complete response).

Safety. Incidence of adverse events was similar in the TDX and in the chemotherapy-treated patient group. However, dose reduction was needed more frequently in the chemotherapy group than in the TDX treated patient group; discontinuation was needed more in TDX-treated than in chemotherapy-treated patient group, serious and fatal drug-related adverse events were more common in TDX-treated than in chemotherapy-treated patients.

Therefore, despite the non-negligible adverse events, the results of this trial indicate that overall TDX represents a valid therapeutic option for hormone receptor-positive HER2-negative BC patients at early treatment stages, even in those showing very low HER2 expression.

Reference. Trastuzumab Deruxtecan after Endocrine Therapy in Metastatic Breast Cancer. *A. Bardia, X. Hu, R. Dent, K. Yonemori, C.H. Barrios, J.A. O'Shaughnessy, H. Wildiers, J.-Y. Pierga, Q. Zhang, C. Saura, L. Biganzoli, J. Sohn, S.-A. Im, C. Lévy, W. Jacot, N. Begbie, J. Ke, G. Patel, and G. Curigliano, for the DESTINY-Breast06 Trial Investigators**. The new england journal of medicine 2024. doi: 10.1056/NEJMoa2407086.

What's new from IEO Researchers?

Tucatinib and trastuzumab for the treatment of advanced metastatic breast cancer: Results of a phase II clinical trial.

Overexpression of HER2 protein (*human epidermal growth factor receptor 2*), resulting in the hyperactivation of the downstream cell processes, leads to uncontrolled cell proliferation, inhibition of cell death, and metastases. Therefore, HER2 is a validated actionable target and, indeed, several studies demonstrated clinical efficacy of HER2-targeted drugs, in breast, gastric and colorectal cancer.

Since about 2-5% of breast tumors have mutations in the HER2 gene, especially in advanced stage disease, in the frame of the phase II basket trial "SGNTUC-019" (aimed at evaluating the efficacy of the HER2-targeted drug tucatinib in combination with trastuzumab, in advanced solid tumors), researchers, including Giuseppe Curigliano –vice-scientific director of IEO, head of the division of New Drug Development for Innovative Therapies, and co-director of the New Drugs program- evaluated safety and efficacy of the treatment combining Tucatinib (a tyrosine kinase inhibitor that is highly selective for HER2), in combination with trastuzumab, in patients with HER2-mutated metastatic breast cancer.

Although overall preliminary, and requiring further investigation in larger patient cohorts or at longer follow-up, these results propose the tucatinib-trastuzumab combination treatment in HER-mutated metastatic breast cancer patients in which multiple prior therapy lines had failed, likely offering an additional therapeutic option. Indeed, the combination of tucatinib and trastuzumab proved effective in reducing tumor size and increasing survival; two patients even showed complete response, encouraging further investigations.

Finally, although the search for biomarkers of response did not allow to draw final conclusions, their results demonstrated the

Tucatinib. Tyrosine-kinase inhibitor. By binding to the HER2 receptor, tucatinib inhibits HER2 kinase activity, thus blocking the downstream signaling. Differently from other small molecules, tucatinib is highly specific for HER2. Preclinical in vitro studies showed that tucatinib only kills HER2-overexpressing cells; in vivo, this inhibitor is able by itself to induce tumor regression in different tumor types, either administered alone or in combination with other drugs. In particular, tucatinib displayed enhanced antitumor activity if administered in combination with trastuzumab or docetaxel.

Trastuzumab. HER2-directed antibody. By selectively binding the extracellular dominion of HER2, it inhibits the downstream signaling and, hence, cancer cell proliferation. Trastuzumab showed efficacy in slowing down tumor progression when administered alone, and its antitumoral efficacy significantly increased when administered in combination with chemotherapy.

Basket trial. Differently from the conventional tumor-specific clinical studies, in which patients are enrolled and administered specific experimental therapeutic protocols on the basis of their own tumor type, basket trials are clinical trials in which patients with the same molecular alteration are treated with the same targeted drug independently from the tumor type. Basket trials are a novel clinical experimental design, evolved, in a precision medicine perspective, with the diffusion of next-generation sequencing approaches, together with the "umbrella trials" in which, instead, different types of molecular therapies are tested (on the basis of specific biomarkers) on patients with the same tumor type.

feasibility of next-generation sequencing (NGS)-based analyses, of both blood and tumor tissue, for the identification of oncogenic mutations in the HER2 gene, which may aid in distinguishing potential responders.

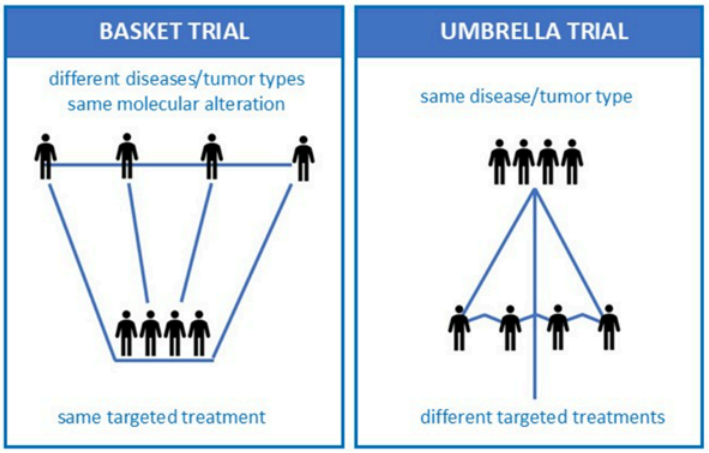
TELL ME MORE!

The trial enrolled 31 patients with HER2-mutated metastatic breast cancer who had progressed during previous therapy lines. Patients received Tucatinib and trastuzumab (some patients –HR-positive– also received fulvestrant).

Efficacy. Signs of response to treatment manifested early after therapy initiation; 73% of the enrolled patients displayed reduced tumor size; 42% of the patients showed objective response rate (as assessed by the investigator), and two patients even achieved complete response. Despite the relatively short follow-up (median follow-up duration, 15 months), the response to treatment appeared durable: Indeed, both progression-free survival (PFS) and overall survival (OS) evaluation enabled to predict PFS at 12 months of 45% of the patients, and OS at 12 months of 74% of the patients (specifically, median PFS was 9.5 months and median OS was 21.1 months).

Safety. The combination treatment was well tolerated even in heavily pre-treated patients. Overall, 26% of patients had serious treatment-related adverse events, 10% of which related to tucatinib. Anyway, adverse events led to discontinuation of tucatinib administration in two patients only, although in some cases dose reductions were needed. None of the patients enrolled died because of the treatment (although 11 of them died because of disease progression).

Biomarker analysis. Once tested for safety and efficacy, they performed a discovery analysis searching for potential biomarkers to identify putative responders. NGS-based analyses of blood or tumor tissue allowed for the identification of a wide range of different HER2 mutations (mostly in the tyrosine kinase domain or in the extracellular domain) and co-occurring mutations (frequently in CDH1, PIK3CA) in responders. Although the number of patients was too low to draw solid conclusions regarding biomarkers, their analyses demonstrated the employability of NGS in tumor tissue and blood for HER2 mutation testing and patient stratification.



Reference. Tucatinib and trastuzumab in HER2-mutated metastatic breast cancer: a phase 2 basket trial. *Alicia F C Okines, Giuseppe Curigliano, Nobumasa Mizuno, Do-Youn Oh, Andree Rorive, Hatem Soliman, Shunji Takahashi, Tanios Bekaii-Saab, Mark E Burkard, Ki Y Chung, Philip R Debruyne, Jenny R Fox, Valentina Gambardella, Marta Gil-Martin, Erika P Hamilton, Bradley J Monk, Yoshiaki Nakamura, Danny Nguyen, David M O'Malley, Alexander B Olawaiye, Bhavana Pothuri, Martin Reck, Kazuki Sudo, Yu Sunakawa, Cedric Van Marcke, Evan Y Yu, Jorge Ramos, Sherry Tan, Mark Bieda, Thomas E Stinchcombe, Paula R Pohlmann*. Nat Med 2025. doi: 10.1038/s41591-024-03462-0.

What's new from IEO Researchers?

Improving clinical response in ER+/HER2- breast cancer patients with immunotherapy - results of a phase III clinical trial.

The current standard approach for the treatment of ER+/HER2- breast cancer patients, in the early disease stage, is chemotherapy (either neoadjuvant or adjuvant) and prolonged endocrine therapy, with or without targeted therapy. However, the clinical outcome of these patients is highly heterogeneous.
Since immunotherapy has shown remarkable results in triple-negative breast cancer (TNBC) patients, and some ER+/HER2- patients exhibit –similar to TNBC– high abundance of immune cells within the tumor mass, the authors reasoned that immunotherapy (specifically, nivolumab, a drug targeting PD1 protein on immune cells and interfering with its binding with PDL1 protein on cancer cells), by boosting antitumor immune activity, may improve patient response to the current standard neoadjuvant therapy. Therefore, in the frame of a phase III clinical trial, researchers, including Giuseppe Curigliano –vice-scientific director of IEO, head of the division of New Drug Development for Innovative Therapies, and co-director of the New Drugs program– evaluated safety and efficacy of the administration of immunotherapy (nivolumab) along with chemotherapy (anthracycline and taxane-based), in the neoadjuvant setting, for the treatment of early stage ER+/HER2- patients.
Their results provide crucial information for the treatment of this breast cancer subtype for which remarkable –though so far unsuccessful– effort has aimed at improving response to treatment. The addition of the immunotherapeutic nivolumab to chemotherapy significantly improved patient response to therapy (rate of complete clinical response). The effects were particularly evident in patients with PDL1-expressing tumors and in those with higher numbers of intratumor immune cells; for these patients, this approach may represent the new standard of care. Although the results were not sufficient to draw conclusions about survival, based on results of previous trials, increased response is likely to correlate with increased survival (event-free survival), strongly encouraging further studies aimed at assessing whether the improved clinical response can be translated into increased survival for all ER+/HER2- patients or only for the PDL1-expressing population.

Neoadjuvant therapy is the administration of a given therapy *before* the main treatment. For instance, before surgery, neoadjuvant therapy is administered to patients in order to reduce tumor size and allow for better clinical outcome. Conversely, **adjuvant therapy** is administered *after* the main treatment in order to decrease the probability of disease recurrence.
Nivolumab. Monoclonal antibody that, by specifically binding PD1 protein expressed on immune cells, prevents the interaction of PD1 with PDL1 protein expressed on tumor cells. By interfering with PD1-PDL1 interaction, nivolumab interferes with a physiological mechanism usually acting as a "brake" to the excessive activity of the immune system against PDL1-expressing cells. By removing this inhibition, immune cells are more active and thus more capable of killing PDL1-expressing tumor cells.

TELL ME MORE!

Patients were enrolled in 221 care centers in 31 countries. One group of 257 patients received, in the neoadjuvant setting, chemotherapy and immunotherapy, while a second group of 253 patients received chemotherapy and a placebo.

Efficacy. The administration of immunotherapy along with chemotherapy improved the clinical response: They observed a higher percentage of patients achieving complete clinical response or minimal residual disease at the end of treatment. Survival (event-free survival) appeared similar in the two patient groups; however, the duration of the follow-up was not sufficient to draw statistically relevant conclusions.

Due to the nivolumab targeting of the PD1/PDL1 axis, the authors explored the possibility of identifying responders on the basis of the degree of intratumoral immune cell infiltration and PDL1 expression levels. The effect was more evident among PDL1-positive tumors and those displaying high intratumoral immune infiltration as compared to PDL1-negative tumors exhibiting poor intratumor immune infiltration. Nevertheless, in-depth investigation of treatment efficacy in relation to biomarker expression revealed that patients with tumors PDL1-negative or lacking intratumor immune cells also showed some benefits.

Safety. The safety profile was overall consistent with what was previously known, and did not prevent patients from undergoing surgery after neoadjuvant treatment: Both patients receiving chemotherapy and immunotherapy and those receiving chemotherapy and placebo experienced adverse events; however, serious, treatment-related adverse events, in some cases leading to treatment discontinuation, were more frequent in the combination therapy group (than in the placebo). In some patients, immune-modulating drugs were needed.

Reference. Neoadjuvant nivolumab and chemotherapy in early estrogen receptor-positive breast cancer: a randomized phase 3 trial. Sherene Loi, Roberto Salgado, Giuseppe Curigliano, Roberto Iván Romero Díaz, Suzette Delaloge, Carlos Ignacio Rojas García, Marleen Kok, Cristina Saura, Nadia Harbeck, Elizabeth A Mittendorf, Denise A Yardley, Alberto Suárez Zaizar, Facundo Rufino Caminos, Andrei Ungureanu, Joaquin G Reinoso-Toledo, Valentina Guarneri, Daniel Egle, Felipe Ades, Misena Pacius, Aparna Chhibber, Rajalakshmi Chandra, Raheel Nathani, Thomas Spires, Jenny Qun Wu, Lajos Pusztai, Heather McArthur. Nat Med 2025. doi: 10.1038/s41591-024-03414-8.

What's new from IEO Researchers?

Combining immunotherapy and chemotherapy for the treatment of endometrial cancer.

Nicoletta Colombo

Preclinical studies suggested that combining immunotherapy with chemotherapy may offer a synergistic approach to treating endometrial cancer. Moreover, clinical studies showed the efficacy of the combined treatment with anti-PDL1 immunotherapy and chemotherapy in cervical cancer, triple-negative breast cancer, non-small cell lung cancer. However, there was limited data regarding the potential efficacy of this combination therapy in endometrial cancer, where the standard first-line treatment is represented by (carboplatin–paclitaxel) chemotherapy, with an overall survival of approximately three months.
In the recent international phase III AtTEnd trial led by Nicoletta Colombo, Director of the IEO Gynecology Program and Associate Professor at the University of Milano-Bicocca, the authors demonstrated efficacy and safety of the combined treatment with anti-PDL1 immunotherapy (that is, the immune checkpoint inhibitor atezolizumab) and (carboplatin/paclitaxel-based) chemotherapy for the treatment of advanced/recurrent endometrial cancer.
Notably, this combination treatment showed significant efficacy in patients with defective DNA damage repair systems (specifically mismatch repair (MMR) deficiency, MMRd). This therapeutic approach significantly improved progression-free survival and overall survival, paving the way to a new standard of care in this setting.

TELL ME MORE!

The AtTEnd trial enrolled 551 patients in 89 care centers in 11 different countries. Patients were randomized to either receive standard chemotherapy (carboplatin and paclitaxel) and anti-PDL1 immunotherapy or chemotherapy and a placebo. AtTEnd is the first large phase III study,

with long follow-up (median: 28.3 months), showing an improved progression-free survival and overall survival of the atezolizumab-chemotherapy combined treatment in advanced/recurrent endometrial cancer patients. The greater efficacy of the treatment observed in the DNA repair system-deficient patient subgroup is in agreement with previous studies showing efficacy of anti-PD1 immunotherapy and chemotherapy in such patients. Indeed, MMR system deficiency may lead to an increased mutation burden, generating neoantigens that are recognized by the immune system, thereby boosting its antitumor activity. However, further analyses in the different patient subgroups, considering factors such as race and microbiota (which has been increasingly shown to influence immunotherapy outcomes), will be important before drawing definitive conclusions on the efficacy of the combined treatment in MMR-proficient tumors.

Reference. Atezolizumab and chemotherapy for advanced or recurrent endometrial cancer (AtTEnd): a randomised, double-blind, placebo-controlled, phase 3 trial. *Nicoletta Colombo, Elena Biagioli, Kenichi Harano, Francesca Galli, Emma Hudson, Yoland Antill, Chel Hun Choi, Manuela Rabaglio, Frederic Marmé, Christian Marth, Gabriella Parma, Lorena Fariñas-Madrid, Shin Nishio, Karen Allan, Yeh Chen Lee, Elisa Piovano, Beatriz Pardo, Satoshi Nakagawa, John McQueen, Claudio Zamagni, Luis Manso, Kazuhiro Takehara, Giulia Tasca, Annamaria Ferrero, Germana Tognon, Andrea Alberto Lissoni, Mariacristina Petrella, Maria Elena Laudani, Eliana Rulli, Sara Uggeri, M Pilar Barretina Ginesta; AtTEnd study group*. Lancet Oncol 2024. doi: 10.1016/S1470-2045(24)00334-6.

What's new from IEO Researchers?

How does the gut microbiota modulate patient response to immunotherapy?

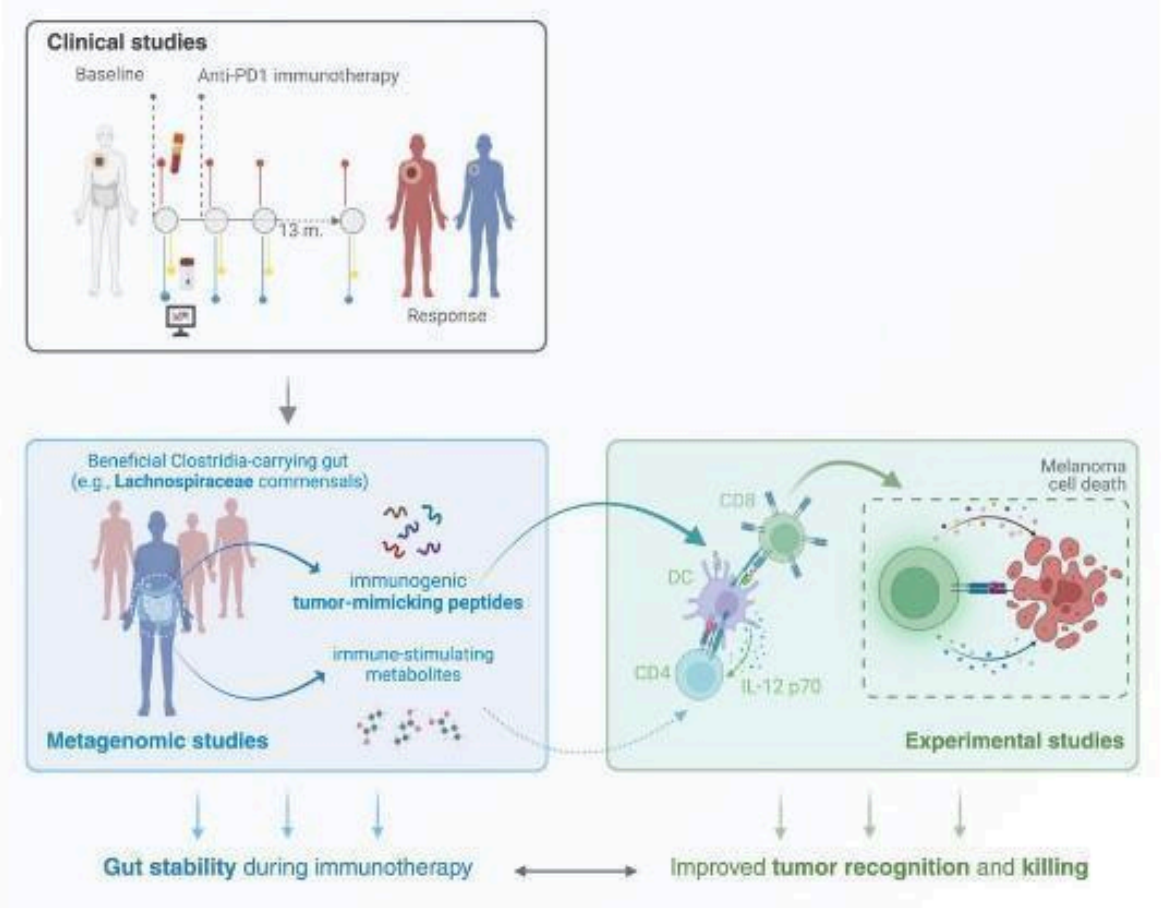


Figure from Macandog, Catozzi et al., Cell Host & Microbe 2024 (an open access article under the CC BY NC ND licence.)

Although immune checkpoint inhibitor (ICI)-based therapy significantly improved melanoma patient clinical outcome, many patients do not respond to the treatment. Previous studies have shown both a different gut microbiome composition of immunotherapy-treated patients who responded to treatment (responders) as compared to those who did not (non-responders), as well as the modulatory role of the gut microbiota, which has been shown to enhance therapy efficacy in patients receiving fecal microbiota transplantation (FMT) from either responders or healthy donors (e.g. see Newsletter n.1), in some cases even leading to an effect in refractory patients.

To get further insights into the mechanisms through which the gut microbiota modulates the response to therapy, in a recent paper by Macandog, Catozzi et al., the authors, supervised by Luigi Nezi –Group leader of the department of experimental oncology of IEO–, profiled the gut microbiome of melanoma patients before and during anti-PD1 therapy, and analyzed the matched blood samples –to describe the effects at systemic level–, showing that a "stable" gut microbiota –in terms of composition, over time during therapy– correlates with response to therapy. Moreover, the researchers identified microbial genes –stably present from the beginning to the exit of the patients from the study– endowed with an extraordinary prognostic power which, because of their presence in patients already before therapy, enabled stratification of cancer

patients in responders or non-responders, in this study as well as across multiple international patient cohorts.

Nezi Lab

Finally, they experimentally demonstrated that some bacterial peptides derived from these microbial genes were highly similar to some tumor proteins and can be used to potentiate the response to immunotherapy. Therefore, on one side, this work demonstrates the importance of a longitudinal analysis of the gut microbiome, which enabled the identification of gut microbiota species linked to response to therapy, whereas, instead, the analyses performed before therapy only did not. Moreover, the parallel analysis of microbiome and blood samples allowed to correlate microbiome, systemic effects at the immune system level and response to therapy. On the other side, this study identifies factors (peptides) of the microbiome able to potentiate the response to therapy, delving into the mechanistic details linking microbiome/immune system underlying the mutual interaction between the two.

TELL ME MORE!

Firstly, the authors analyzed how ICI administration changed the gut microbiome composition and how this was related to the response to therapy. To do so, they profiled fecal samples of advanced melanoma patients, before and during ICI therapy, at multiple timepoints. They assumed that the species being the most prevalent across the samples collected at different time points were likely to be biologically relevant. Under this assumption, they found that while differentially abundant species in CR as compared to nonCR were not stably abundant at the different time points (that is, not necessarily a species that was more abundant in CR than in nonCR at the first time point remained more abundant at the next time point and so on), the

most prevalent species in CR remained stably prevalent at the different time points analyzed, suggesting their biological relevance. Notably, also in other (4 of the 9 analyzed) patient cohorts, in which previous studies found limited reproducibility of microbiome-based signatures, the same CR-associated species identified in this work maintained their enrichment in responders, both before and during therapy.

Since the gut microbiota influences the immune system and the immune system influences the microbiota, once analyzed the gut microbiome, they analyzed blood samples (namely, white blood cell count and inflammatory soluble factor quantification) during therapy, in order to delve into the systemic immune effect, and link them with the gut microbiome features. Neutrophil to lymphocyte ratio (NLR) was low (that is, low neutrophil count and high lymphocyte count) during therapy in CR as compared to nonCR. Moreover, the analysis of soluble inflammatory molecules before and during therapy revealed that the presence in the blood of one specific inflammatory molecule (IL-12p70) was associated with CR, while four others (CX3CL1/FRACTALKINE, IL-7, IL-8, HGF) were associated with nonCR. The levels of these inflammatory molecules were also associated with NLR. Furthermore, the CR-associated cytokine levels correlated with the microbiome species that remained stable across timepoints in CR, whereas the association of the four nonCR-associated cytokines with microbiome species was less strong, highlighting, unlike the CR, the high variability of the nonCR microbiota composition.

Overall, these results demonstrate the clinical relevance of the longitudinal profiling of the gut microbiota, which enabled patient stratification into (complete) responders and non (complete) responders whereas, instead, previous microbiota signatures analyzing only microbiota composition at baseline, failed.

The functional analysis revealed some microbial pathways consistently enriched before and during therapy in the microbiota of CR (namely, the pathways active in the species overrepresented in CR gut microbiota), such as those involved in flagellar assembly and bacterial chemotaxis, as well as starch and sucrose metabolism. The analysis highlighted a much less clear situation in nonCR. Strikingly, the gene families associated with these bacterial pathways were a much better predictor of response to ICI than bacterial species themselves, enabling effective stratification of CR and nonCR patients across multiple international patient cohorts, at baseline. Because of the clinical relevance of their new finding, the researchers next focused on the mechanistic investigation of the underlying differences between CR and nonCR microbiota unraveled. The flagellin genes (involved in flagellar assembly) were the most overrepresented in the gut microbiota of CR. Notably, these proteins are known to be involved in the modulation of the immune system. In particular, the authors observed that among the flagellin proteins of the Lachnospiraceae (FLach), some (three) were very similar (in terms of sequence and in silico-predicted 3D structure) to melanoma tumor-associated antigens (TAAs). Interestingly, these TAAs were preferentially expressed on tumors of CR rather than those of nonCR. They delved into the relation between FLach proteins and the immune system, and showed a greater reactivity against FLach peptides of T cells isolated (before ICI therapy) from the blood of CR melanoma as compared to nonCR. In addition, FLach peptides elicited a stronger expansion *in vitro* of tumor infiltrating T cells (TIL) from melanoma tumors, demonstrating the presence of an intratumoral FLach-directed immunity that could be potentially exploited for therapeutic purposes. Indeed, TILs expanded *in vitro* with FLach peptides demonstrated higher killing ability against matching melanoma organoids, providing evidence that these peptide can potentiate antigen-specific antitumor immune response against melanoma.

Therefore, while it has been previously proposed that the gut microbiota influences the immune

The NLR, namely the ratio between the neutrophil and lymphocyte count in the peripheral blood, is usually considered a marker of disease, the manifestation of an inflammatory status, in different pathological conditions, including infections and cancer. Several studies suggest that the NLR can be employed as a prognostic marker in different tumor types, proposing its potential exploitation in a clinical setting.

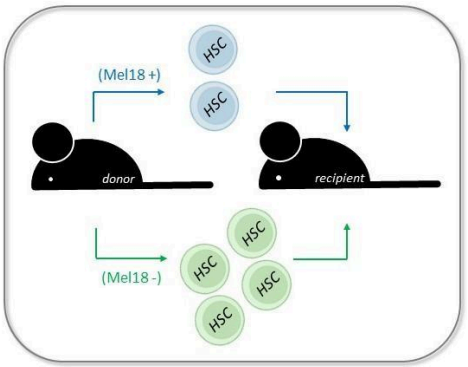
system through the release of metabolites, these results strongly suggest that it may also happen through the direct modulation of the immune system by gut bacterial proteins, such as the flagellins. Studies are ongoing to exploit FLach peptides to develop innovative predictive and therapeutic tools to improve life perspectives of patients with melanoma and, potentially, other solid tumors.

Reference. Longitudinal analysis of the gut microbiota during anti-PD-1 therapy reveals stable microbial features of response in melanoma patients. *Angeli D G Macandog, Carlotta Catozzi, Mariaelena Capone, Amir Nabinejad, Padma P Nanaware, Shujing Liu, Smita Vinjamuri, Johanna A Stunnenberg, Serena Galiè, Maria Giovanna Jodice, Francesca Montani, Federica Armanini, Ester Cassano, Gabriele Madonna, Domenico Mallardo, Benedetta Mazzi, Salvatore Pece, Maria Tagliamonte, Vito Vanella, Massimo Barberis, Pier F Ferrucci, Christian U Blank, Marlene Bouvier, Miles C Andrews, Xiaowei Xu, Laura Santambrogio, Nicola Segata, Luigi Buonaguro, Emilia Cocorocchio, Paolo A Ascierto, Teresa Manzo, Luigi Nezi*. Cell Host Microbe 2024. doi: 10.1016/j.chom.2024.10.006.

What's new from IEO Researchers?

Targeting the Polycomb complex to enhance ex vivo expansion of hematopoietic stem cells before transplantation. A new approach?

All blood cells derive from a subset of cells called hematopoietic stem cells (HSCs). These cells ensure blood homeostasis (that is, the correct equilibrium, at any time, among all different cell components of the blood) throughout life and play a key role during hematopoietic stem cell transplantation (HSCT). Indeed, these cells are endowed with the so-called self-renewal ability, namely the ability, when transplanted in patients, to proliferate and differentiate into virtually all blood cell types. However, the mechanisms underlying HSC self-renewal are not completely understood and an in-depth understanding may allow to precisely interfere with such mechanisms and improve the efficacy of transplant.



In a recent paper, researchers –including Diego Pasini, Group leader of the dept of experimental oncology of IEO, in the frame of a collaboration with Yan Liu's group at the Northwestern University (Chicago)– demonstrated the key role of Polycomb complex proteins –playing a key role in cell physiology, acting as repressors of gene expression when needed (see Tamburri et al., Mol Cell 2024. Newsletter n.5)– in HSC self-renewal. In particular, the authors demonstrated that the Mel18 subunit of the Polycomb complex inhibits HSC self-renewal and its loss leads to an increase of self-renewal, promoting proliferation, both in *in vitro* assays and in *in vivo* preclinical models.

Their findings appear clinically relevant in the context of HSCT. Indeed, although this approach is widely used in the clinical setting for the treatment of leukemia patients, a significant hurdle is represented by the limited ability, with the currently available methods, to expand HSCs isolated from the donors before transplantation into a recipient. This work proposes an approach, to be further investigated, leveraging the repressive activity of Polycomb complex, and in particular the Mel18 subunit, to improve *ex vivo* expansion of donor HSCs: The strictly regulated removal –or inhibition– of Mel18 may indeed enable to enhance HSC expansion prior to transplantation, thus facilitating the whole manufacturing process linked with HSCT, and reducing the associated costs.

TELL ME MORE!

Polycomb group protein Bmi1 is known to be critically involved in HSC self-renewal: Bmi1 loss impairs HSC self-renewal, whereas Bmi1 overexpression promotes self-renewal. In this work, by exploiting conditional murine models devoid of another subunit of the Polycomb complex, Mel18, specifically in the hematopoietic system, the authors showed that Mel18 loss altered the blood cell composition (e.g. resulting in increased numbers of monocytes, reduced numbers of neutrophils, platelets and red cells) and the numbers of HSCs (leading to an increase of LT-HSC, MPP and Lin-, while not affecting ST-HSCs, cKIT+ cells). Indeed, they observed that Mel18 loss promoted HSC self-renewal, both in *in vitro* (colony forming) assays and *in vivo* (increasing the engraftment of Mel18-deficient HSCs into irradiated recipient mice), and promoted cell cycle progression. The increased self-renewal of Mel18-deficient HSCs correlated with changes in gene expression, including genes linked to HSC (such as Hoxb4, whose ectopic

expression is known to increase HSC *ex vivo* expansion). The altered gene expression profile induced by Mel18 loss was due to a change in chromatin accessibility, which ultimately resulted in the increased self-renewal of HSCs. Consistently, ectopic re-expression of Mel18 caused reduced Hoxb4 gene expression and self-renewal, indicating that Mel18 negatively regulated HSC maintenance, hence its loss promoted HSC expansion. Moreover, Mel18 deficiency resulted in the lost repression of cell cycle-related genes –such as cyclin dependent kinases, E2F1, and Myc– thus promoting cell cycle progression. Notably, Mel18 loss affected different genes as compared to those affected by Bmi1, indicating that the two proteins play distinct roles and act through the regulation of different genes. Mechanistically, Mel18 –which is part of the Polycomb repressive complex 1– works by repressing gene expression through the addition of ubiquitin molecules on histone proteins and, indeed, in Mel18-deficient cells histone ubiquitination (H2AK119ub1) levels were altered. In particular, among the genes showing reduced ubiquitination at histone 2 upon Mel18 loss, they found cell cycle-related genes.

Reference. Polycomb group protein Mel18 inhibits hematopoietic stem cell self-renewal through repressing the transcription of self-renewal and proliferation genes. *Wenjie Cai, Xicheng Liu, Sergio Barajas, Shiyu Xiao, Sasidhar Vemula, Hongxia Chen, Yuxia Yang, Christopher Bochers, Danielle Henley, Sheng Liu, Yuzhi Jia, Michelle Hong, Tiffany M Mays, Maegan L Capitano, Huiping Liu, Peng Ji, Zhonghua Gao, Diego Pasini, Jun Wan, Feng Yue, Leonidas C Platanias, Rongwen Xi, Sisi Chen, Yan Liu.* Leukemia 2025. doi: 10.1038/s41375-024-02462-w.

What if biased measurements led to a misinterpretation? Employing uneven testing conditions the results of protein-protein interaction studies.

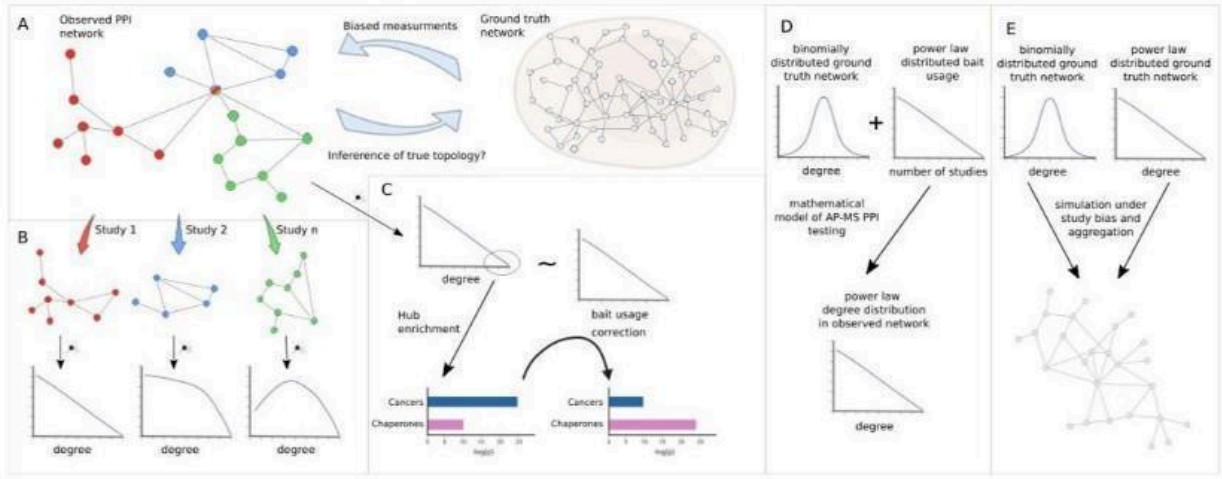


Figure from Blumenthal, Lucchetta et al., eLife2024 (an open access article under the CC BY licence)

A disease can be due to alterations in protein expression levels or sequence, as well as to the abnormal interaction with other proteins. For instance, in leukemia, mutations in the NPM1 protein trigger the aberrant interaction with ARF protein (absent in a physiological context), causing NPM1 delocalization and degradation, in turn leaving MDM2 protein free to induce p53 ubiquitination and degradation, thus preventing p53-dependent cell death in response to stimuli and hence the oncogenic transformation. This mechanism is an example of tumorigenesis induced by changes in protein-protein interaction.

Computational tools have been increasingly used in biomedical research and, for instance, they can be leveraged to characterize protein-protein interaction networks and predict the involvement of a given protein in a disease. A previous work, involving IEO researchers, reported the development of a user-friendly web application –Robust-web– to identify, within an existing network, protein-protein interaction subnetworks enriched in proteins with certain properties, such as disease-relevance (Sarkar et al., Bioinformatics 2023. Newsletter n.1).

To describe the probability of individual proteins to form a specific number of interactions, scientists usually use mathematical models relying on the Power Law; that is, a mathematical function describing the degree distribution of protein networks (in other words, the Power Law fits the number of interactions each protein tends to form).

However, in a recent paper by Blumenthal, Lucchetta et al., research involving Martin Schaefer -Group Leader at the department of experimental oncology of IEO-, show that only some (less than 30%)

protein-protein interaction networks could be actually described by the Power Law (that is, only in some protein networks the Power Law actually accurately approximates the degree distribution). This is most likely due to the fact that the protein-protein interaction networks obtained by aggregating data from different studies are profoundly influenced by proteins that have been more frequently tested (see Newsletter n.1), introducing a bias, in combination with high error rates associated with protein-protein interaction detection methods. In this paper, the authors mathematically and empirically demonstrated that, due to this bias, even networks that could not be described by the Power Law appear as if they do. As a result, protein-protein interaction network descriptions can be flawed: Some proteins can result as being interacting with other proteins while they do not and, *viceversa*, some protein-protein interactions actually occurring inside the cell may remain undetected. Biologically speaking, by leading to the misidentification of protein interactions, some proteins may result as being linked with some diseases even though they are not. Instead, when correcting this bias, the Power Law fails to describe the protein-protein networks.

A bias affects the results. How to overcome that?

"A putative way to overcome this issue –suggests Martin Schaefer– may be to systematically investigate protein-protein interaction without *a priori* hypothesis/knowledge on the importance of a given protein in a cell (as it has been the focus of the ongoing HuRI project; Luck et al., Nature 2020). On the other hand, the bias may be actually amplified by the aggregation of data from single studies and analyses performed on non-aggregated studies, normalizing the degree distribution, or algorithmically taking protein testing frequencies into account may actually contribute to reducing the bias, which would allow to better understand cellular networks without overstating the role of heavily studied proteins."

What is the consequence of this finding for future biological studies? First, these results call for caution when interpreting interactome data. Indeed, the mentioned bias appears to be particularly relevant in the context of cancer research. The interactome refers to the ensemble of protein-protein interactions occurring within a cell and unveiling such interactions is critical to understand the cell behavior. Indeed, each protein exerts its function not working by itself inside the cell, but as part of a network. Detecting abnormalities in protein interactions –that is, differences as compared to the physiological status– can reveal novel targetable mechanisms of disease.
Moreover, these findings indicate that the emergence of the Power Law properties for biological networks cannot be biologically explained, as it has been previously suggested, by the gene duplication model (according to this model, the Power Law describes protein-protein interaction networks considering that proteins originating from a gene duplication may retain the same set of interactions with other proteins, while mutations may allow for new interactions).

Reference. Emergence of power law distributions in protein-protein interaction networks through study bias. *David B Blumenthal #, Marta Lucchetta #, Linda Kleist, Sándor P Fekete, Markus List, Martin H Schaefer*. Elife 2024. doi: 10.7554/eLife.99951.

News, initiatives and events from the IEO world!

Two IEO research projects awarded at the Seed4Innovation.

Two research projects developed at the IRCCS European Institute of Oncology (IEO) have been awarded during the 4th edition of the Seed4Innovation program, promoted by Fondazione UNIMI and the University of Milan. The STRIVE project, coordinated by prof. Stefano Santaguida –IEO group leader and associate professor at the University of Milan– received the Proof of Concept grant by the University of Milan and is aimed at developing new therapies for breast cancer, through the identification of specific surface markers on cancer cells.

The EPIKIN project, developed by the team headed by Gioacchino Natoli –IEO group leader–, with the contribution of Francesco Gualdrini and Sara Polletti, obtained the Proof of Concept grant of the *Camera di Commercio Milano Monza Brianza Lodi*. EPIKIN is an innovative platform for the genomic profiling of kinase inhibitors, sustaining the advancement and clinical development of these molecules and paving the way to new therapeutic options.

IEO-TT, the IEO technology transfer office, headed by Marzia Fumagalli and with the contribution of Claudia Iavarone, Senior Specialist at IEO-TT, enthusiastically supported the research teams in any phase of the Seed4Innovation program. IEO-TT will continue to offer its own support in the following acceleration phases, with the goal of transforming these innovative ideas into reality.

A special acknowledgement to Fondazione UNIMI, the University of Milan and the mentors' network coordinated by Antonio Alessandrino, who supported the researchers in this path, and in particular to the mentors Lavinia Capuana, Fabrizio Bacchi, Paolo Luperto e Gianluca Sferrazza, who played a crucial role in strengthening the vision and potential impact of IEO projects.

News, initiatives and events from the IEO world!

Innovation day 2024.

On December 19th 2024, at the IRCCS European Institute of Oncology (IEO), took place the Innovation day 2024, an event organized to celebrate 30 years of research, progress and commitment of our institute in improving health and well-being.

The scientific supervisor of the day was Dr Marzia Fumagalli, head of IEO-TT, the IEO technology transfer office, who moderated the activities together with professionals of the field, researchers and innovators, who discussed current challenges, new opportunities and emerging technologies that are shaping the future of cancer medicine, from digital solutions to personalized treatments.

The opening speech was given by Mauro Melis, CEO of IEO, followed by the Scientific Director Roberto Orecchia and the director of the department of experimental oncology, Pier Giuseppe Pelicci, who highlighted how innovation is the engine of our future.

Francesco Cerruti, Director of the Italian Tech Alliance, underlined challenges and opportunities for innovation in Italy, showing positivity about the coming years.

Two roundtables fueled the debate. The first one, chaired by Saverio Minucci –director of a research unit at IEO–, Nicola Fusco –director of the IEO Division of Pathological Anatomy–, Holger Neecke –Amministratore Delegato di Tethis SpA– and Marcella Origgi –Early Innovation Partner-Italy at Johnson & Johnson.

The second roundtable was chaired by Marzia Fumagalli, focused on entrepreneurship and startup creation in the life sciences; Fabio Bianco –Scientific Director of Bio4Dreams–, Roberto Chiarle –Director of the IEO Division of Hemolymphopathology–, Federica Draghi –Founder and Partner in charge of XGEN Venture SGR–, Saverio Minucci, and Alessandro Tozzi –Founding partner and CEO of Endostart Srl– contributed to the discussion
Ennio Tasciotti –Director of the Human Longevity Program of the IRCCS San Raffaele in Rome– highlighted how mistakes can significantly inspire innovation, offering food for thoughts.
The day ended with a session of business pitch presentations of some innovative projects developed in IEO, chaired by Claudia Iavarone –Senior Specialist at IEO-TT. Among the speakers, Salvatore Pece, Lucilla Titta, Gioacchino Natoli, Pier Paolo Di Fiore and Luigi Nezi.

News, initiatives and events from the IEO world!

Excellence in urology: IEO receives the *Bollino Azzurro* from the Fondazione Onda ETS.

IEO received from the Fondazione Onda ETS the *Bollino Azzurro*, which values excellence in prevention, diagnosis, treatment and rehabilitation in the uro-andrologic field. This achievement is the result of work and commitment of IEO personnel, united by the will to improve patients' quality of life and promote greater awareness about male health.

News, initiatives and events from the IEO world!

Christmas Gala for Research – 30th Anniversary: 415.000 euros for the IEO-MONZINO Foundation.

Last November, in the *Spazi Rubattino Studio | R56*, in Milan, took place the traditional Christmas Gala for Research of the Fondazione IEO-MONZINO ETS, the charity dinner dedicated to donors, physicians, researchers and supporters. This year, the gala was particularly special, celebrating 30 years of IEO and IEO-Monzino Foundation; the latter one, founded in 1994 to support research at IEO and Monzino.
Headed by Patrizia Sandretto Re Rebaudengo, over the years the foundation expanded its activity, accompanying its clinical-scientific commitment with activities aimed at increasing awareness on the importance of prevention, as well as oncological and cardiovascular research.
The evening was possible thanks to the support of several partners, and the collaboration of Carla and Alessandro Cordiano's Ploom PR, specialized in global communication and events. What really made the event unique was the generosity of the 700 guests who, by joining the dinner and the silent auction, contributed to achieve an extraordinary result for medical research: 415.000 euros.
The foundation expresses its deepest gratitude to all those who made the event possible, from the generosity of guests to the sponsors – Allianz, Gilead Kite, LabAurelia Group, MIAMO, Pellegrini and REPLY -, from the organizing staff to artists, who donated their time and talent for a noble cause. The event was the successful result of commitment for the common good.

IEO MEMBERS - LET'S GET TO KNOW THEM BETTER.

Elena Dal Zotto, grant officer.

Elena - The Grant Wolf (with a weakness for Eggplant Parmigiana).

I'm a Grants Officer on a mission: to turn the brilliant ideas of basic and clinical research PIs into funded projects. I'm the secret weapon that allows them to focus on science, while I navigate calls, budgets and financial reports. Think Mr. Wolf from Pulp Fiction, but with spreadsheets.

In 4 years at IEO I've perfected my skills in the pre and post-award of European funded projects, surviving tight deadlines, crazy calls and savage audits. I participate in the EU-LIFE network, coordinating the Grants & Funding Strategies working group. I am in my sweet spot at European tables, because I like to know which way the wind is blowing in research funding policies.

I don't wear a white coat, but I have an analytical mind, a strong belief in scientific rigor (even though I read the horoscope) and a passion for creative environments that pose intellectual challenges. I have a degree in Economics and Development. My choice to focus on emerging economies reflects my interest in international contexts with a future yet to be written.

I'm a judo black belt, a discipline that has taught me the importance of continuous improvement and making the best use of energy. In my free time, I recharge by practicing yoga and organizing hikes. I love to travel, because with a backpack on my shoulder I express my best version, the one most open to discovery and change.

My goal is to continue to support scientific research, helping researchers advance in cancer knowledge and improve people's lives, one grant at a time.

THE BRIEFING

A glance through recent papers from IEO researchers, and from the whole scientific community.

What else is new from IEO researchers?

A panel of experts to standardize best practices of microbiome testing for clinical implementation. Despite the growing scientific evidence indicating the influence of the gut microbiota in health and disease and its potential exploitation in the clinic-diagnostic setting, the broad employment of microbiota-based diagnostic tools is currently limited, due to a number of factors. In this review, the authors elaborated best practices for the implementation of microbiome-based diagnostics in clinical routine.

Serena Porcari, Benjamin H Mullish, Francesco Asnicar, Siew C Ng, Liping Zhao, Richard Hansen, Paul W O'Toole, Jeroen Raes, Georgina Hold, Lorenza Putignani, Christian Lodberg Hvas, Georg Zeller, Omry Koren, Hein Tun, Mireia Valles-Colomer, Maria Carmen Collado, Monika Fischer, Jessica Allegretti, Tariq Iqbal, Benoit Chassaing, Josbert Keller, Simon Mark Baunwall, Maria Abreu, Giovanni Barbara, Faming Zhang, Francesca Romana Ponziani, Sam P Costello, Sudarshan Paramsothy, Dina Kao, Colleen Kelly, Juozas Kupcinskas, Ilan Youngster, Francesco Franceschi, Sahil Khanna, Maria Vehreschild, Alexander Link, Flavio De Maio, Edoardo Pasolli, Aitor Blanco Miguez, Patrizia Brigidi, Brunella Posteraro, Franco Scaldaferri, Mirjana Rajilic Stojanovic, Francis Megraud, Peter Malfertheiner, Luca Masucci, Manimozhiyan Arumugam, Nadeem Kaakoush, Eran Segal, Jasmohan Bajaj, Rupert Leong, John Cryan, Rinse K Weersma, Robert Knight, Francisco Guarner, Fergus Shanahan, Patrice D Cani, Eran Elinav, Maurizio Sanguinetti, Willem M de Vos, Emad El-Omar, Joel Dorè, Julian Marchesi, Herbert Tilg, Harry Sokol, Nicola Segata, Giovanni Cammarota, Antonio Gasbarrini, Gianluca Ianiro.
Lancet Gastroenterol Hepatol 2024. PMID: 39647502.

Identifying diet-related differences in the gut microbiota. By analyzing the gut microbiome of over 20.000 individuals from 5 different independent human cohorts, the authors found that the gut microbiome profile distinguished the three diet regimens: Omnivore, vegetarian and vegan. Their results highlighted that the omnivore microbiome (strongly driven by a red meat-containing diet) negatively correlated with cardiometabolic health, while vegan diet-related microbiome profile positively correlated with cardiometabolic health.

Gloria Fackelmann, Paolo Manghi, Niccolò Carlino, Vitor Heidrich, Gianmarco Piccinno, Liviana Ricci, Elisa Piperni, Alberto Arrè, Elco Bakker, Alice C Creedon, Lucy Francis, Joan Capdevila Pujol, Richard Davies, Jonathan Wolf, Kate M Bermingham, Sarah E Berry, Tim D Spector, Francesco Asnicar, Nicola Segata.
Nat Microbiol 2025. PMID: 39762435.

Modulating the gut microbiome through a non-industrialized diet provides health benefits. The authors showed that a specific nutritional regimen, recapitulating key features of non-industrialized diets, along with the administration of a specific gut bacterium (*Limosilactobacillus reuteri*), despite reducing gut microbiota diversity, restores microbiome features altered by industrialization, beneficially modifies the microbiota-derived blood metabolites associated with non-communicable diseases, and provides cardiometabolic benefits, indicating that dietary interventions can rescue the dysbiotic gut microbiome associated with chronic pathologies, thus representing a potentially effective nutrition-based therapeutic approach.

Fuyong Li, Anissa M Armet, Katri Korpela, Junhong Liu, Rodrigo Margain Quevedo, Francesco Asnicar, Benjamin Seethaler, Tianna B S Rusnak, Janis L Cole, Zhihong Zhang, Shuang Zhao, Xiaohang Wang, Adele Gagnon, Edward C

Deehan, João F Mota, Jeffrey A Bakal, Russell Greiner, Dan Knights, Nicola Segata, Stephan C Bischoff, Laurie Mereu, Andrea M Haqq, Catherine J Field, Liang Li, Carla M Prado, Jens Walter. Cell 2025. PMID: 39855197.

Cancer-preventive strategies in the diet - a review. In this review, the authors report the state of the art regarding the potential of dietary restriction as a cancer-preventive measure, describing the biochemical pathways involved, as well as the current preclinical and clinical studies.
Greta Caprara, Rani Pallavi, Shalini Sanyal, Pier Giuseppe Pelicci.
Nutrients 2025. PMID: 39940361.

A novel Fusobacterium species isolated from colorectal cancer tissue. The authors isolated and sequenced F. sphaericum sp. nov., a new bacterial species from colon cancer tissue, phenotypically and genetically different, yet with a metabolic and antibiotic resistance profile similar to other Fusobacterium species. In vitro, the new species associates with colon cancer epithelial cells and displays immunomodulatory properties. It is rarely found in the human stool of both healthy and cancer patients.
Martha A Zepeda-Rivera, Yannick Eisele, Alexander Baryiames, Hanrui Wu, Claudia Mengoni, Gianmarco Piccinno, Elsa F McMahon, Kaitlyn D LaCourse, Dakota S Jones, Hans Hauner, Samuel S Minot, Nicola Segata, Floyd E Dewhirst, Christopher D Johnston, Susan Bullman.
Gut Microbes 2025. PMID: 39722539.

The oral microbiome of irradiated head and neck cancer patients. Microenvironmental stimuli (such as salivary flow, drinking, chewing) contribute to shaping the oral microbiome. Previous studies have shown that the unique microbiome in the periodontal pocket of the human gingiva, interacting with the host and playing a key role in preserving oral health, may cause oral diseases (such as gingivitis, periodontitis) in case of shift form healthy/physiological to unbalanced conditions, and, in the long term, can lead to the establishment of pathogens possibly contributing to non-oral diseases (e.g. Inflammatory Bowel Disease, Alzheimer's Disease, arthritis) thus threatening human health. The authors reported the characterization of the oral microbiota of patients with head and neck cancer who received radiotherapy, providing useful knowledge for future studies aimed at defining the potential involvement of oral microbiota in oral toxicity (such as radiation-related caries, affecting about one third of irradiated patients) after radiation therapy.
Romualdo M Filho, Claudia Mengoni, Julia S Bruno, Eduardo R Fregnani, Nicola Segata, Anamaria A Camargo, Vitor Heidrich.
Microbiol Resour Announc 2024. PMID: 39745461.

Describing a potential source of (pathogenic) human microbiome strains - the canine dental plaque microbiome. Through shotgun metagenomic sequencing and metagenomic assembly, the authors described the -mostly unknown- dog plaque microbiome, highlighting that, despite the poor overlap at the species-level, human and dog plaque microbiomes appear more conserved at the family level, supporting the use of dogs as models of human periodontal diseases and highlighting the role of dog plaque microbiome as potential source of pathogenic human microbiome strains.
Vitor Heidrich, Gloria Fackelmann, Milka Malesevic, Federica Armanini, Hrituraj Dey, Claudia Mengoni, Nemanja Stanisavljevic, Goran Vukotic, Nicola Segata.
NPJ Biofilms Microbiomes 2025. PMID: 39966419.

The use of cannabis in medical oncology - The TASMAN study. Through this study, the authors highlighted the need for clear regulations and standardized guidelines to support the broader employment of cannabis for pain management in medical care, to improve the quality of life of cancer patients.
Dario Trapani, Sara J Nidhamalddin, Sara Gandini, Marco Filetti, Sara C Altuna, Ambra Carnevale Schianca, Angelica Petrillo, Shilpa M Murthy, Fabio Girardi, Jacques B Bezuidenhout, Khalid El Bairi, Pasquale Lombardi, Shah Z Khan, Csongor G Lengyel, Andreas Seeber, Sadaqat Hussain, Fahmi U Seid, Essam Elfaham, Andrew O Odhiambo, Yakup Coskun, Habeeb S Baker, Arman R Chowdhury, Armando Genazzani, Gennaro Daniele, Giampiero Porzio, Giuseppe Curigliano, Raffaele Giusti.
Eur J Cancer 2024. PMID: 39647216.

Histone modification in cancer: The NSD proteins. NSDs (nuclear receptor-binding SET domain) are proteins that, through the epigenetic modification of histones, regulate key cell processes. In this review, the authors provide an overview of the role of NSD proteins in cancer progression, including virus-induced tumors, and the potential of their therapeutic targeting.
Lavinia Ghiani, Susanna Chiocca.
Tumour Virus Res 2024. PMID: 39645166.

Polycomb in non-dividing quiescent cells. The authors showed that, differently from proliferating cells, in quiescent cells, Polycomb complex is mostly constituted by the less enzymatically active (EZH1-PRC2 and cPRC1) subunits, making these cells refractory to PRC2 inhibitors, and thus raising concerns regarding the efficacy of PRC2-targeted drugs in cancers containing high numbers of non-proliferating cells.
Rachel McCole, James Nolan, David M Reck, Craig Monger, Samantha Rustichelli, Eric Conway, Gerard L Brien, Cheng Wang, Orla Deevy, Hannah K Neikes, Frances M Bashore, Aoibhinn Mooney, Richard Flavin, Elisabeth Vandenberghe, Sarena F Flanigan, Diego Pasini, Chen Davidovich, Michiel Vermeulen, Lindsey I James, Evan Healy, Adrian P Bracken.
Cell Rep 2025. PMID: 39799569.

Impaired mitochondrial function in fibromyalgia patients. The authors found that patients affected by Fibromyalgia syndrome display impaired mitochondrial function as compared to healthy controls, and patients with a more severe syndrome have reduced mitochondrial function as compared to those showing a less severe symptomatology. Moreover, their results suggest that the mitochondrial dysfunction appeared to involve the musculoskeletal system rather than the central nervous system.
Chiara Macchi, Andrea Giachi, Isabella Fichtner, Silvia Pedretti, Piercarlo Sarzi Puttini, Nico Mitro, Alberto Corsini, Massimiliano Ruscica #, Roberta Gualtierotti #.
Sci Rep 2024. PMID: 39632893.

Tracing the evolution of West Nile Virus (WNV). West Nile virus (WNV) is a mosquito-borne pathogen that can infect humans. Among the European countries, Italy is one of those most hit by the infection. Thanks to the creation of a database comprising all available WNV genomes, the authors traced the evolution of the virus, shedding light on its dynamics in Italy and providing a reference for future studies.
Andrea Silverj, Giulia Mencattelli, Federica Monaco, Federica Iapaolo, Liana Teodori, Alessandra Leone, Andrea Polci, Valentina Curini, Marco Di Domenico, Barbara Secondini, Valeria Di Lollo, Massimo Ancora, Annapia Di Gennaro, Daniela Morelli, Maria Gabriella Perrotta, Giovanni Marini, Roberto Rosà, Nicola Segata, Omar Rota-Stabelli, Annapaola Rizzoli, Giovanni Savini; West Nile Virus Working Group.
Epidemiol Infect 2024. PMID: 39620707.

LEfSer – an R-based computational tool for metagenomic biomarker discovery and visualization. In this paper, the authors present an improved, R-based version of the Python-based LEfSe algorithm for biomarker discovery using metagenomic data, displaying improved accuracy, performance and reproducibility when used in human and murine metagenomic datasets.
Asya Khleborodova, Samuel D Gamboa-Tuz, Marcel Ramos, Nicola Segata, Levi Waldron, Sehyun Oh.
Bioinformatics 2024. PMID: 39585730.

Analyzing cell cycle in non-adherent cells. By combining specialized surfaces for cell attachment, a fluorescent sensor for cell cycle analysis (FUCCI) and a machine learning algorithm, the authors developed a fully automated, freely available tool for the analysis of cell cycle phase duration in non-adherent cells.
Kourosh Hayatigolkhatmi #, Chiara Soriani #, Emanuel Soda #, Elena Ceccacci, Oualid El Menna, Sebastiano Peri, Ivan Negrelli, Giacomo Bertolini, Gian Martino Franchi, Roberta Carbone, Saverio Minucci, Simona Rodighiero.
Elife 2024. PMID: 39576677.

GM1 ganglioside potential for the treatment of Rett syndrome. The authors show that the hydrophilic oligosaccharide chain of GM1 restores synaptogenesis and reduces mitochondrial oxidative stress in in vitro cultures derived from preclinical models of Rett syndrome, and ameliorates Rett syndrome symptoms in in vivo preclinical models, suggesting its potential exploitation for the treatment of Rett syndrome.

Maria Fazzari, Giulia Lunghi, Emma Veronica Carsana, Manuela Valsecchi, Eleonora Spiombi, Martina Breccia, Silvia Rosanna Casati, Silvia Pedretti, Nico Mitro, Laura Mauri, Maria Grazia Ciampa, Sandro Sonnino, Nicoletta Landsberger, Angelisa Frasca, Elena Chiricozzi.
Int J Mol Sci 2024. PMID: 39519108.

Oral microbiome and autism. Through the metagenomic analysis of oral microbiomes -exploiting over 7000 salivary samples subjected to whole genome sequence-, the authors found that children diagnosed with autism spectrum disorders (ASD) have a different oral microbiome composition as compared to control siblings; 108 differentially abundant species distinguished ASD subjects from controls. The results strongly support further studies exploring the potential of oral microbiome-based biomarkers in ASD.
Paolo Manghi, Michele Filosi, Moreno Zolfo, Lucas G Casten, Albert Garcia-Valiente, Stefania Mattevi, Vitor Heidrich, Davide Golzato, Samuel Perini, Andrew M Thomas, Simone Montalbano, Samuele Cancellieri, Levi Waldron, Jacob B Hall, Simon Xu, Natalia Volfovsky, LeeAnne Green Snyder, Pamela Feliciano, Francesco Asnicar, Mireia Valles-Colomer, Jacob J Michaelson, Nicola Segata, Enrico Domenici.
Nat Commun 2024. PMID: 39528484.

Metabolo-epigenetic characterization of induced cardiomyocytes. The authors characterized the histone post-translational modification landscape and the metabolome of adult fibroblasts-derived cardiomyocytes, unraveling epigenetic and mitochondrial metabolism changes accompanying cardiomyocyte differentiation. In vitro and in vivo metabolic/dietary modulation improved fibroblast-cardiomyocyte conversion, suggesting the potential exploitation of metabolic/epigenetic modulation to improve cardiac regenerative strategies.
Francisco Santos, Magda Correia, Rafaela Dias, Bárbara Bola, Roberta Noberini, Rita S Ferreira, Diogo Trigo, Pedro Domingues, José Teixeira, Tiziana Bonaldi, Paulo J Oliveira, Christian Bär, Bruno Bernardes de Jesus, Sandrina Nóbrega-Pereira.
Aging Cell 2024. PMID: 39540462.

Primary tumor ablation in metastatic renal cancer. The authors found that local (either cryo- or heat-based) ablation of the primary tumor in metastatic renal cell carcinoma patients resulted in increased survival as compared to those who did not undergo local treatment, while no statistically significant differences in survival were observed between patients undergoing tumor ablation and surgery.
Lukas Scheipner, Reha-Baris Incesu, Simone Morra, Andrea Baudo, Letizia Maria Ippolita Jannello, Carolin Siech, Mario de Angelis, Anis Assad, Zhe Tian, Fred Saad, Shahrokh F Shariat, Alberto Briganti, Felix K H Chun, Derya Tilki, Nicola Longo, Luca Carmignani, Ottavio De Cobelli, Martin Pichler, Sascha Ahyai, Pierre I Karakiewicz.
Urol Oncol 2024. PMID: 39537442.

Neoadjuvant chemo-immunotherapy for the treatment of breast cancer - a review. In this review, the authors offer an overview and discuss the current available evidence related to factors predicting response to chemo- and chemo-immunotherapy in the neoadjuvant (before surgery) setting for the treatment of early stage breast cancer patients.
Chiara Corti, Busem Binboğa Kurt, Beyza Koca, Tasnim Rahman, Fabio Conforti, Laura Pala, Giampaolo Bianchini, Carmen Criscitiello, Giuseppe Curigliano, Ana C Garrido-Castro, Sheheryar K Kabraji, Adrienne G Waks, Elizabeth A Mittendorf, Sara M Tolaney.
Cancer Treat Rev 2024. PMID: 39571402.

Neoadjuvant chemotherapy and neoadjuvant chemoimmunotherapy in breast cancer patients - a review. The authors collected and discussed translational and clinical evidence related to predictors of efficacy in early stage breast cancer patients treated with either neoadjuvant chemotherapy or neoadjuvant chemoimmunotherapy. Available data indicate that treatment efficacy may be related to a number of tumor features (before treatment), although the heterogeneity of the studies has prevented so far the integration of all these factors into a single predictive model.

Chiara Corti, Busem Binboğa Kurt, Beyza Koca, Tasnim Rahman, Fabio Conforti, Laura Pala, Giampaolo Bianchini, Carmen Criscitiello, Giuseppe Curigliano, Ana C Garrido-Castro, Sheheryar K Kabraji, Adrienne G Waks, Elizabeth A Mittendorf, Sara M Tolaney.
Cancer Treat Rev 2025. PMID: 39571402.

Guidelines for the clinical management of breast cancer patients at risk of brain metastases. The authors provide guidelines for the clinical management of HER2-positive metastatic breast cancer patients -who are at significant risk of developing brain metastases- and for trial design, including aspects related to screening, assessment of symptoms, treatment efficacy and prevention.
Volkmar Müller, Thomas Bachelot, Giuseppe Curigliano, Evandro de Azambuja, Julia Furtner, Jens Gempt, Barbara Alicja Jereczek-Fossa, Katarzyna J Jerzak, Emilie Le Rhun, Carlo Palmieri, Gabriella Pravettoni, Cristina Saura, Rupert Bartsch.
Cancer Treat Rev 2024. PMID: 39612906.

Guidelines for the clinical management of HER2-positive metastatic breast cancer in real world scenarios. The poor available scientific evidence makes it difficult to define the optimal treatment approach in the third line setting for HER2-positive breast cancer patients. In this review, the authors provide guidance for clinicians for the treatment of HER2-positive breast cancer patients in real world, challenging (that is, for which the optimal therapeutic approach has not been defined yet) scenarios (including elderly patients, those with brain-only metastases, ERBB2-mutant disease).
Rupert Bartsch, David Cameron, Eva Ciruelos, Carmen Criscitiello, Giuseppe Curigliano, Francois P Duhoux, Theodoros Foukakis, Joseph Gligorov, Nadia Harbeck, Nathalie LeVasseur, Alicia Okines, Frederique Penault-Llorca, Volkmar Müller.
Cancer Treat Rev 2024. PMID: 39580869.

Surgery in small intestine neuroendocrine tumors: Yes or no? In this work, the authors showed that patients affected by small intestine neuroendocrine tumors with metastases and primary tumor-related symptoms had worse overall survival as compared to non-metastatic and non-symptomatic patients; therefore, they may benefit from the surgical resection of the primary tumor, as symptoms may evolve and negatively affect prognosis after surgery.
Maria Danieli, Uberto Fumagalli Romario, Davide Radice, Simonetta Pozzi, Francesca Spada, Luigi Funicelli, Nicola Fazio, Emilio Bertani.
Ann Surg Oncol 2024. PMID: 39627636.

A journey in the physical principles of thoracic surgery. In this review, the authors describe the physics principles at the basis of the most common thoracic surgery approaches, providing basic –physics-centered– understanding, for thoracic surgeons, of concepts such as radiosterilization, electrosurgery, fluid dynamics, endoscopic techniques, diffusion principles, and laser technologies, with the ultimate goal of fostering the interdisciplinary collaboration for the continuous technological innovation as well as for the correct use of such advanced technologies.
Luca Bertolaccini, Virginia Piva, Antonio Mazzella, Monica Casiraghi, Marco Maria Jacopo Felisi, Lorenzo Spaggiari.
J Clin Med 2024. PMID: 39597896.

Therapy-related adverse events in patients carrying pathogenic germline BRCA variants. In the frame of a retrospective study, the authors found that, unlike the platinum-based or radiation-based treatment, the administration of PARP inhibitors as maintenance therapy did not result into more hematologic-related adverse events in patients with advanced ovarian cancer carrying a pathogenic germline BRCA variant as compared to non-carriers.
Carmine Valenza, Eleonora Nicolò, Marta Mongillo, Dario Trapani, Jalissa Katrini, Laura Boldrini, Luca Boscolo Bielo, Grazia Castellano, Lorenzo Guidi, Gloria Pellizzari, Jacopo Villa, Silvia Derio, Mariateresa Lapresa, Federica Gigli, Gabriella Parma, Emanuela Omodeo Salè, Enrico Derenzini, Giuseppe Curigliano, Nicoletta Colombo.
Oncologist 2024. PMID: 39607864.

Computational models to predict the effect of gene fusions on inhibitor efficacy - the case of ALK inhibitors. Lung cancer carrying fusion of the ALK protein can be treated with ALK-targeted inhibitors. However, how the ALK fusion partner may influence treatment outcome is unknown. In this study, the authors performed an *in silico* study to predict the protein resulting from the striatin-ALK fusion, proposing a putative mechanism underlying the lack of response to lorlatinib of cells carrying this gene alteration and the parallel short-duration response to alectinib, and indicating that AI tools may be used to predict the effects of gene fusions, especially when rare gene partners are involved.
Massimo Barberis, Alessandra Rappa, Filippo de Marinis, Giuseppe Pelosi, Elena Guerini Rocco, Yinxiu Zhan, Guido Tiana.
Transl Lung Cancer Res 2024. PMID: 39830770.

p53 mutations in breast cancer tissue sections - Leveraging machine learning. The authors reported the development of a deep learning algorithm to predict p53 mutational status by employing H&E-stained breast cancer tissue sections. The tool displayed greater precision and efficiency as compared to currently available methods, offering a novel tool for precision oncology, to reduce intra- and inter-observer variability in the diagnostic setting. Further validation will allow to integrate this tool in the clinical routine.
Chiara Frascarelli, Konstantinos Venetis, Antonio Marra, Eltjona Mane, Mariia Ivanova, Giulia Cursano, Francesca Maria Porta, Alberto Concardi, Arnaud Gerard Michel Ceol, Annarosa Farina, Carmen Criscitiello, Giuseppe Curigliano, Elena Guerini-Rocco, Nicola Fusco.
Comput Struct Biotechnol J 2024. PMID: 39678362.

Profiling mismatch repair (MMR) alterations. MMR deficiency (preventing a correct repair of DNA errors) is frequent in cancer and represents a biomarker of disease, exploitable for the clinical management of cancer patients. However, the current assays for MMR detection only evaluate expression levels of the MRR system proteins, thus not taking into account potential mutations of these proteins. In this work, by means of an integrated analysis of genomic data of over 23.000 patients and 11 tumor types, the authors described the frequency, spectrum and distribution of MMR mutations in solid tumors, demonstrating a high degree of heterogeneity, the different patient clinical outcome in relation to MMR mutations, and the need for more accurate and comprehensive testing approaches to refine the current diagnostic strategies.
Konstantinos Venetis, Chiara Frascarelli, Luca Boscolo Bielo, Giulia Cursano, Riccardo Adorisio, Mariia Ivanova, Eltjona Mane, Virginia Peruzzo, Alberto Concardi, Mariachiara Negrelli, Marianna D'Ercole, Francesca Maria Porta, Yinxiu Zhan, Antonio Marra, Dario Trapani, Carmen Criscitiello, Giuseppe Curigliano, Elena Guerini-Rocco, Nicola Fusco.
Eur J Cancer 2025. PMID: 39827722.

Breast cancer risk in patients carrying a pathogenic/likely pathogenic mutation - a review. Women carrying pathogenic or likely pathogenic (P/LP) variants in specific genes have higher risk of developing breast cancer. Anyway, the percentage of individuals carrying these alterations and actually developing the disease during their lifetime is variable, and depends on several factors. Carefully estimating this risk for each individual would enable the design of personalized preventive strategies. In this review, the authors discuss risk factors, risk assessment, and potential preventive strategies to be adopted by women carrying P/LP variants, including lifestyle modification, chemoprevention, intensified surveillance, and risk-reducing surgeries.
Eliza Del Fiol Manna, Davide Serrano, Laura Cazzaniga, Sara Mannucci, Cristina Zanzottera, Francesca Fava, Gaetano Aurilio, Aliana Guerrieri-Gonzaga, Matilde Risti, Mariarosaria Calvello, Irene Feroce, Monica Marabelli, Cecilia Altemura, Lucio Bertario, Bernardo Bonanni, Matteo Lazzeroni.
Genes (Basel) 2025. PMID: 39858629.

A conservative approach to occult breast cancer treatment. Occult breast cancer is a rare condition in which a metastatic mass is detected at the level of the axillary without detection of the primary breast cancer lesion. In the frame of this retrospective study, the authors evaluated the safety of a conservative

approach for the management of occult breast cancer, omitting axillary dissection even in case of sentinel lymph node biopsy positivity in selected patients who exhibited complete pathological response after neoadjuvant chemotherapy.
Elisa Vicini, Viviana Galimberti, Maria Cristina Leonardi, Sabrina Kahler-Ribeiro-Fontana, Andrea Polizzi, Salvatore Petitto, Eleonora Pagan, Vincenzo Bagnardi, Emilia Montagna, Matteo Cavallone, Pietro Caldarella, Mattia Intra, Paolo Veronesi.
Breast Cancer Res Treat 2025. PMID: 39776333.

Treating secondary AML. Previous clinical studies have shown greater efficacy of fludarabine combinations (FLAG-Ida) and CPX-351 as compared to conventional chemotherapy for the treatment of secondary AML. In this work, the authors analyzed the effect of such treatments on the minimal residual disease (MRD), showing a higher complete remission rate and higher probability of negative MRD in CPX-351-treated as compared to FLAG-Ida-treated, an overall increased survival in CPX-351-treated patients, and higher tolerability.
Carola Riva, Paola Minetto, Maria Chies, Chiara Vernarecci, Nicoletta Colombo, Sara Rosellini, Alessia Parodi, Elisabetta Tedone, Enrico Carminati, Clara Nurra, Francesco Puglisi, Michela Frello, Elena Maio, Beatrice Ferro, Giada Zecchetti, Giuseppina Fugazza, Paolo Nozza, Michele Cea, Roberto Massimo Lemoli, Fabio Guolo.
Hematol Oncol 2025. PMID: 39635952.

Venetoclax, intensive chemo-immunotherapy and hematopoietic stem cell transplantation for the treatment of Richter syndrome. Richter syndrome (RS, an aggressive disease characterized by aggressive lymphoma in chronic leukemia patients) represents an unmet clinical need. The authors report two cases of RS patients who achieved long-term complete remission upon treatment with Venetoclax (BCL2 inhibitor) and intensive chemo-immunotherapy before receiving hematopoietic stem cell transplantation, with no major toxicity or complications, indicating the feasibility of this approach.
Enrico Derenzini, Alessandro Cignetti, Valentina Tabanelli, Daniela Gottardi, Elvira Gerbino, Anna Vanazzi, Simona Sammassimo, Alessio Maria Edoardo Maraglino, Federica Melle, Giovanna Motta, Daniela Malengo, Emanuela Omodeo Salè, Lisa Bonello, Rocco Pastano, Stefano Pileri, Fabrizio Carnevale Schianca, Corrado Tarella.
Hematol Rep 2024. PMID: 39728005.

A review and meta-analysis to help clinicians in the treatment of male breast cancer. Through this analysis, the authors assessed the benefits, in terms of overall survival, of including radiation therapy after surgery in the therapeutic approach of male breast cancer patients, highlighting the need for further investigation aimed at identifying the patient subgroups who may benefit the most from radiation therapy.
Riccardo Ray Colciago, Valentina Lancellotta, Maria Carmen De Santis, Elisabetta Bonzano, Fiorenza De Rose, Eliana La Rocca, Bruno Meduri, Nadia Pasinetti, Agnese Prisco, Alessandra Gennari, Trine Tramm, Serena Di Cosimo, Nadia Harbeck, Giuseppe Curigliano, Philip Poortmans, Icro Meattini, Pierfrancesco Franco.
Crit Rev Oncol Hematol 2024. PMID: 39454738.

In a review, current evidence on the employment of metronomic chemotherapy for breast cancer treatment. By collecting and analyzing current evidence regarding efficacy and toxicity related to the use of metronomic chemotherapy (namely, the administration of low dose chemotherapy over prolonged time periods as compared to the conventional, high dose administration for a limited time) for the treatment of breast cancer patients, the authors suggest it may not be the optimal therapeutic approach in all settings, and, when available, other approaches with robustly proven, greater efficacy should be prioritized.
Elena Battaiotto, Simeone d'Ambrosio, Dario Trapani, Giuseppe Curigliano.
Clin Breast Cancer 2024. PMID: 39627044.

Antibody-drug conjugates (ADC) - the state of the art. In this review, the authors discuss latest advancements and future challenges related to the employment of ADC in cancer therapy, covering aspects such as ADC technologies, different payloads (chemotherapy, toxins, radionuclides) and

integration with PROTACS for the precise degradation of the target, as well as the potential of ADC-based combination therapies.
Davide Izzo, Liliana Ascione, Lorenzo Guidi, Renato Maria Marsicano, Chrysanthi Koukoutzeli, Dario Trapani, Giuseppe Curigliano.
Ther Adv Med Oncol 2025. PMID: 39759830.

Treating HER2-positive breast cancer with Trastuzumab duocarmazine antibody-drug conjugate. In the frame of the phase III TULIP trial, the authors showed that although the risk of disease progression in advanced stage HER2-positive breast cancer patients who progressed under previous treatment with either targeted therapy or trastuzumab emtansine was lower under treatment with the HER2-targeted antibody-drug conjugate Trastuzumab duocarmazine (T-Duo), tolerability was affected by ocular toxicity that frequently led to treatment discontinuation.
Nicholas Turner, Cristina Saura, Philippe Aftimos, Evelyn van den Tweel, Mayke Oesterholt, Norbert Koper, Marco Colleoni, Emilie Kaczmarek, Kevin Punie, Xinni Song, Anne Armstrong, Giulia Bianchi, Agostina Stradella, Sylvain Ladoire, Joline Si Jing Lim, Nathalie Quenel-Tueux, Tira J Tan, Santiago Escrivá-de-Romaní, Joyce O'Shaughnessy; TULIP Trial Investigators.
J Clin Oncol 2025. PMID: 39442070.

PI3K inhibitors as novel therapeutic options for breast cancer - a review. In this review the authors discuss the state of the art and future challenges -mainly due to the related toxicity- associated with emerging therapeutics targeting the PI3K pathway for the treatment of breast cancer.
Riccardo Asnaghi, Gabriele Antonarelli, Elena Battaiotto, Grazia Castellano, Lorenzo Guidi, Davide Izzo, Paola Zagami, Dario Trapani, Giuseppe Curigliano.
Expert Opin Pharmacother 2025. PMID: 39846444.

Combining Imlunestrant with abemaciclib for the treatment of advanced breast cancer patients. In the frame of a phase III clinical trial, the authors demonstrated that i) imlunestrant (a ER degrader, whose administration results in sustained ER inhibition) alone increased progression-free survival in ESR1-mutated ER-positive, HER2-negative breast cancer patients, and ii) administering imlunestrant in addition to abemaciclib significantly increased patient progression-free survival as compared to imlunestrant alone in patients with relapsing (after CDK4/6 inhibitor therapy) advanced breast cancer, independently from ESR1 mutations.
Komal L Jhaveri, Patrick Neven, Monica Lis Casalnuovo, Sung-Bae Kim, Eriko Tokunaga, Philippe Aftimos, Cristina Saura, Joyce O'Shaughnessy, Nadia Harbeck, Lisa A Carey, Giuseppe Curigliano, Antonio Llombart-Cussac, Elgene Lim, María de la Luz García Tinoco, Joohyuk Sohn, André Mattar, Qingyuan Zhang, Chiun-Sheng Huang, Chih-Chiang Hung, Jorge Luis Martinez Rodriguez, Manuel Ruíz Borrego, Rikiya Nakamura, Kamnesh R Pradhan, Christoph Cramer von Laue, Emily Barrett, Shanshan Cao, Xuejing Aimee Wang, Lillian M Smyth, François-Clément Bidard; EMBER-3 Study Group.
N Engl J Med 2024. PMID: 39660834.

Radiotherapy-based treatment of metastatic breast cancer. The authors showed that breast cancer patients with metastases in other (less than five) organs benefit from stereotactic radiotherapy (SBRT) specifically directed at the metastatic site in addition to the systemic therapy (especially in the HR+/HER2- subgroup).
Monica Milano, Carmine Valenza, Annamaria Ferrari, Sara Gandini, Dario Trapani, Celeste Santoro, Elena Battaiotto, Ambra Carnevale Schianca, Elisa Giordano, Jalissa Katrini, Grazia Castellano, Beatrice Taurelli Salimbeni, Maria Cristina Leonardi, Samantha Dicuonzo, Carmen Criscitiello, Nadia Bianco, Silvia Dellapasqua, Elisabetta Munzone, Giuseppe Curigliano, Marco Colleoni, Barbara Alicja Jereczek-Fossa.
Eur J Cancer 2025. PMID: 39662096.

Prognostic significance of abnormal tumor margins in infiltrating laryngeal tumors. By analyzing a 281-patient cohort, the authors showed that the existence of dysplastic margins after the radical surgical resection of laryngeal infiltrating tumors does not affect patient survival. Therefore, these patients may not need to undergo aggressive post-surgery treatment (associated with poorer quality of life).

Francesco Chu, Francesco Bandi, Giacomo Pietrobon, Marta Tagliabue, Stefano Zorzi, Rita de Berardinis, Pietro Benzi, Mohssen Ansarin.
Am J Otolaryngol 2024. PMID: 39662105.

Retrospective analysis on neuroendocrine gastric cancer patients. The authors retrospectively analyzed clinical data regarding patients with neuroendocrine gastric tumors treated at IEO over a 20-year period. The analysis included 69 patients, either endoscopically or surgically treated, or untreated; clinical, laboratory and histological data were collected. Their analyses showed no cancer-related deaths within the patient cohort and an overall excellent disease prognosis (especially for Type1 tumors).
Davide Ravizza, Mariangela Giunta, Isabella Sala, Vincenzo Bagnardi, Darina Tamayo, Giuseppe de Roberto, Cristina Trovato, Ivana Bravi, Pietro Soru, Margherita Maregatti, Eleonora Pisa, Emilio Bertani, Guido Bonomo, Francesca Spada, Fazio Nicola.
J Neuroendocrinol 2024. PMID: 39191460.

Thromboembolism in neuroendocrine tumors. By investigating the frequency of thromboembolism in neuroendocrine cancer patients, the authors highlighted the rather high frequency of this complication in these patients, especially in advanced stage or poorly differentiated tumors, and further studies may aid in developing optimized prophylactic and therapeutic strategies for this patient population.
Sara Massironi, Lorenzo Gervaso, Fabrizio Fanizzi, Paoletta Preatoni, Giuseppe Dell'Anna, Nicola Fazio, Silvio Danese.
Cancers (Basel) 2025. PMID: 39857994.

Treating late-relapsing ovarian cancer patients - results of a phase III clinical trial. In the frame of the phase III ENGOT-OV41/GEICO 69-O/ANITA clinical trial, the authors showed that the combination of atezolizumab with (platinum-based) chemotherapy, followed by maintenance treatment with niraparib in late-relapsing ovarian cancer patients did not significantly improve neither progression-free survival nor objective response rate.
Antonio González-Martín, María Jesús Rubio, Florian Heitz, René Depont Christensen, Nicoletta Colombo, Toon Van Gorp, Margarita Romeo, Isabelle Ray-Coquard, Lydia Gaba, Alexandra Leary, Luis Miguel De Sande, Coriolan Lebreton, Andrés Redondo, Michel Fabbro, Maria-Pilar Barretina Ginesta, Philippe Follana, J Alejandro Pérez-Fidalgo, Manuel Rodrigues, Ana Santaballa, Renaud Sabatier, Maria José Bermejo-Pérez, Jean-Pierre Lotz, Beatriz Pardo, Gloria Marquina, Luisa Sánchez-Lorenzo, María Quindós, Purificación Estévez-García, Eva Guerra Alía, Luis Manso, Victoria Casado, Stefan Kommoss, Germana Tognon, Stéphanie Henry, Ilan Bruchim, Ana Oaknin, Frédéric Selle.
J Clin Oncol 2024. PMID: 39292975.

Genomic profiling of circulating tumor DNA: Results of the CAPRI 2-GOIM trial. Through the comparison between tumor tissue genomic profiling and circulating tumor DNA genomic profiling in 207 metastatic colorectal cancer patients, the authors showed the feasibility of liquid biopsy-based genomic profiling, the concordance with results of tumor tissue profiling, the greater capability in recapitulating tumor heterogeneity, and the ability to provide clinically relevant information, enabling the identification of additional actionable genomic alterations in about half of RAS/BRAFV600E WT mCRC patients.
D Ciardiello, L Boscolo Bielo, S Napolitano, E Martinelli, T Troiani, A Nicastro, T P Latiano, P Parente, E Maiello, A Avallone, N Normanno, S Pisconti, C Nisi, R Bordonaro, A E Russo, E Tamburini, I Toma, C Lotesoriere, S Vallarelli, M G Zampino, N Fazio, G Curigliano, F De Vita, F Ciardiello, G Martini; CAPRI-2 GOIM study group.
Ann Oncol 2024. PMID: 39214459.

Choosing the right diagnostic test for homologous recombination deficiency (HRD) in ovarian cancer patients: Some guidelines. Through a Delphi consensus study, the authors provide guidelines for the choice of the best diagnostic test to detect HRD in ovarian cancer patients, to aid clinicians in the decision-making process regarding the administration of PARP inhibitors, whose efficacy has been reported to be related to HRD.
Stanislas Quesada, Frédérique Penault-Llorca, Xavier Matias-Guiu, Susana Banerjee, Massimo Barberis, Robert L Coleman, Nicoletta Colombo, Anna DeFazio, Iain A McNeish, Angélica Nogueira-Rodrigues, Ana Oaknin, Sandro Pignata, Éric Pujade-Lauraine, Étienne Rouleau, Aleš Ryška, Nerina Van Der Merwe, Toon Van Gorp, Ignace Vergote, Wilko Weichert, Xiaohua Wu, Isabelle Ray-Coquard, Pascal Pujol; expert consensus group.

Eur J Cancer 2025. PMID: 39693891.

Cemiplimab for the treatment of recurrent cervical cancer - final analysis of the phase III EMPOWER-Cervical 1/GOG-3016/ENGOT-cx9 trial. The authors report the final results of the trial evaluating efficacy of cemiplimab as compared to chemotherapy in patients with recurrent cervical cancer. They showed that, regardless of PDL1 expression, cemiplimab administration increases survival in patients who already progressed under first line chemotherapy, supporting its use as second line treatment.
Ana Oaknin, Bradley J Monk, Andreia Cristina de Melo, Hee Seung Kim, Yong Man Kim, Alla S Lisyanskaya, Vanessa Samouëlian, Domenica Lorusso, Fernanda Damian, Chih-Long Chang, Evgeniy Gotovkin, Shunji Takahashi, Daniella Ramone, Beata Mańkowiak-Matejczyk, Laura Polastro, Eva Maria Guerra Alia, Nicoletta Colombo, Yulia Makarova, Jeffrey C Goh, Kosei Hasegawa, Paulo Mora, Joanna Pikiel, Ratnesh Srivastav, Danny Rischin, Maria Jesús Rubio, Javier Perez, Suk Young Yoo, Bo Gao, Shaheda Jamil, Frank Seebach, Israel Lowy, Melissa Mathias, Matthew G Fury, Krishnansu S Tewari.
Eur J Cancer 2025. PMID: 39798514.

Chemotherapy and PARP inhibitors for the treatment of ovarian cancer. The phase III ARIEL4 trial assessed safety and efficacy of the PARP inhibitor rucaparib in ovarian cancer patients with BRCA1 or BRCA2 mutation who progressed under chemotherapy. The final results of the trial demonstrated that although progression-free survival in PARP inhibitor-treated patients was longer as compared to chemotherapy-treated patients, overall survival was longer in the chemotherapy-treated patient group. Though results on overall survival may be driven by the platinum-resistant patient subpopulation, the data indicate the need for further studies aimed at defining the best treatment -and sequence of administration- approach for these patients.
Amit M Oza, Alla Lisyanskaya, Alexander Fedenko, Andreia Cristina de Melo, Yaroslav Shparyk, Irina Rakhmatullina, Igor Bondarenko, Nicoletta Colombo, Valentyn Svintsitskiy, Luciano Biela, Marina Nechaeva, Domenica Lorusso, Giovanni Scambia, David Cibula, Róbert Póka, Ana Oaknin, Tamar Safra, Beata Mackowiak-Matejczyk, Ling Ma, Daleen Thomas, Kevin K Lin, Karen McLachlan, Sandra Goble, Rebecca Kristeleit.
Lancet Oncol 2025. PMID: 39914419.

Rechallenging platinum-resistant ovarian cancer patients with platinum-based chemotherapy - a retrospective study. In the frame of this single center retrospective study, the authors evaluated the potential efficacy of a treatment with platinum-based chemotherapy in ovarian cancer patients who progressed under treatment with non-platinum based chemotherapy for a platinum-based resistant disease, suggesting the efficacy of this therapeutic approach in selected patients (47% 6-month progression-free survival and 80% disease control rate in a 30-patient cohort).
Carmine Valenza, Marta Mongillo, Maria Vittoria Visconti, Jalissa Katrini, Dario Trapani, Laura Boldrini, Lorenzo Guidi, Alessia Farfalla, Daniela Malengo, Giuseppe Caruso, Silvia Derio, Mariateresa Lapresa, Gabriella Parma, Elena Biagioli, Emanuela Omodeo Salé, Giuseppe Curigliano, Nicoletta Colombo.
Gynecol Oncol 2025. PMID: 39923679.

ER-low and ER-negative breast cancers: Are they really different? The authors compared the immune microenvironment and the gene expression profile of early stage HER2-negative ER-low breast cancer samples, as compared to ER-negative and ER-intermediate tumors, showing that ER-low and ER-negative are biologically and molecularly similar, in agreement with their similar clinical behavior and response to treatment.
Davide Massa, Claudio Vernieri, Lorenzo Nicolè, Carmen Criscitiello, Florence Boissière-Michot, Séverine Guiu, Angélique Bobrie, Gaia Griguolo, Federica Miglietta, Andrea Vingiani, Riccardo Lobefaro, Beatrice Taurelli Salimbeni, Claudia Pinato, Francesca Schiavi, Silvia Brich, Carlo Pescia, Nicola Fusco, Giancarlo Pruneri, Matteo Fassan, Giuseppe Curigliano, Valentina Guarneri, William Jacot, Maria Vittoria Dieci.
J Natl Cancer Inst 2024. PMID: 39083015.

In a review, the state of the art on estrogen receptor degraders. Disrupting estrogen receptor (ER) signaling (by endocrine therapy) is a common strategy to counter HR+ (hormone receptor-positive) breast

cancer progression. However, these tumors often become resistant. In this review, the authors provide an overview of the most recent findings about novel compounds acting through the degradation of ER (resulting in the inhibition of the downstream signaling), covering aspects such as mechanism of action and safety.
Roberta Scafetta, Paola Zagami, Marzia Del Re, Carmen Criscitiello, Antonio Marra, Giuseppe Curigliano. Breast Cancer Res Treat 2025. PMID: 39776334.

Clinical trials: The patients' experience. In this study, the authors analyzed patient experience during the different stages of the clinical trial: Before, during and after enrolment, highlighting the need for further support to patients in the post-trial phase in order to fully understand their stress and improve their well-being as well as to reduce trial dropout rates.
Mariam Chichua, Davide Mazzoni, Chiara Marzorati, Gabriella Pravettoni.
Patient Educ Couns 2025. PMID: 39426006.

Psychological consequences of abnormal findings after cancer screening. In this study, the authors examined the effect of abnormal findings during cancer screening of asymptomatic patients on their mental well being. Their results showed that although overall reporting of abnormal findings did not cause long-term psychological consequences, some subjects with specific personality traits (such as emotional instability and introversion) may experience stronger psychological distress (anxiety).
Lorenzo Conti, Davide Mazzoni, Chiara Marzorati, Roberto Grasso, Derna Busacchio, Giuseppe Petralia, Gabriella Pravettoni.
J Magn Reson Imaging 2025. PMID: 38821883.

Psychological factors affecting response to covid19 vaccine in cancer patients. The authors found that psychological distress, anxiety and depressive symptoms can reduce patient response to covid19 vaccine (specifically, mRNA-based BNT162b2).
Gabriella Rondanina, Tania Buttiron Webber, Oriana D'Ecclesiis, Marco Musso, Irene Maria Briata, Nicoletta Provinciali, Monica Boitano, Matteo Clavarezza, Mauro D'Amico, Carlotta Defferrari, Alberto Gozza, Leonello Innocenti, Alessio Carbone, Martino Oliva, Emanuela Marcenaro, Francesca Filauro, Sara Gandini, Andrea DeCensi.
Cancers (Basel) 2024. PMID: 39682198.

Re-assessing vitamin D supplementation for severe covid-19 disease. Through this meta-analysis, the authors suggest that conclusions drawn from small randomized clinical trials evaluating the potential of vitamin D supplementation in preventing severe covid-19 disease and acute respiratory infections may overestimate the actual benefits of this approach.
Philippe Autier, Giulia Doi, Patrick Mullie, Patrick Vankrunkelsven, Oriana D'Ecclesiis, Sara Gandini.
PLoS One 2025. PMID: 39808630.

Sex-related differences in lung cancer incidence. The authors analyzed the incidence of lung cancer in the north east of Italy (Friuli Venezia Giulia region) in the 1995-2021 period, considering histological subtypes and sex-specific differences. By analyzing data of over 24.000 patients, the analysis highlighted an overall higher incidence in males as compared to females; however, while in the analyzed timeframe incidence rate decreased in men, it increased among women.
Luca Bertolaccini, Claudia Santucci, Carlo La Vecchia, Federica Toffolutti, Giovanni Corso, Lorenzo Spaggiari, Diego Serraino.
Eur J Cancer Prev 2025. PMID: 39932541.

Life expectancy in prostate cancer patients. The authors analyzed life expectancy in rare prostate cancer subtypes as compared to age-matched control, and life expectancy of high-grade prostate cancer patients undergoing treatment as compared to those under surveillance. Their results showed that among the rare prostate cancer subtypes, the neuroendocrine tumors displayed the worst prognosis, independently from disease stage, while the others did not significantly affect life expectancy. Concerning high grade

patients, they appear to have significantly better overall survival when undergoing active treatment rather than surveillance.
Carolin Siech, Mario de Angelis, Letizia Maria Ippolita Jannello, Francesco Di Bello, Natali Rodriguez Peñaranda, Jordan A Goyal, Zhe Tian, Fred Saad, Shahrokh F Shariat, Stefano Puliatti, Nicola Longo, Ottavio de Cobelli, Alberto Briganti, Mike Wenzel, Philipp Mandel, Luis A Kluth, Felix K H Chun, Pierre I Karakiewicz.
Int J Cancer 2024. PMID: 39740082.
Francesco Di Bello, Letizia Maria Ippolita Jannello, Andrea Baudo, Mario de Angelis, Carolin Siech, Zhe Tian, Jordan A Goyal, Massimiliano Creta, Gianluigi Califano, Giuseppe Celentano, Pietro Acquati, Fred Saad, Shahrokh F Shariat, Luca Carmignani, Ottavio de Cobelli, Alberto Briganti, Felix K H Chun, Nicola Longo, Pierre I Karakiewicz.
Prostate 2025. PMID: 39449158.

Life expectancy of adrenocortical carcinoma patients. Through the comparison of adrenocortical carcinoma patients with age- and sex- matched controls, the authors analyzed life expectancy in patients with metastatic, locally advanced and localized tumors, showing that, even in patients with localized tumors, life expectancy is poor (39% lower than the general population).
Letizia Maria Ippolita Jannello, Andrea Baudo, Lukas Scheipner, Mario de Angelis, Carolin Siech, Francesco Di Bello, Jordan A Goyal, Kira Vitucci, Zhe Tian, Stefano Luzzago, Francesco A Mistretta, Matteo Ferro, Fred Saad, Felix K H Chun, Alberto Briganti, Luca Carmignani, Nicola Longo, Ottavio de Cobelli, Gennaro Musi, Pierre I Karakiewicz.
Int Urol Nephrol 2025. PMID: 39129040.

Prognosis of early onset vs late onset colorectal cancer (CRC) – a meta-analysis. Through a meta-analysis, the authors explored potential differences in terms of prognosis between early onset CRC patients (that is, before 50 years of age) as compared to late onset (that is, after 50 years of age) CRC patients. Their results showed that despite the higher percentage of early onset patients diagnosed with later stage disease, no differences in terms of cancer-related survival were observed.
Fabio Carbone, Antonino Spinelli, Davide Ciardiello, Marco Realis Luc, Stefano de Pascale, Emilio Bertani, Nicola Fazio, Uberto Fumagalli Romario.
Eur J Cancer 2025. PMID: 39681013.

Olaparib for the treatment of BRCA-mutated platinum-sensitive relapsed ovarian cancer: Results of the SOLO3 trial. In the frame of a phase III trial, the authors reported the final results regarding overall survival of relapsing, platinum-sensitive ovarian cancer patients carrying BRCA mutations, showing that it was similar in patients treated with olaparib and in those receiving chemotherapy; however, olaparib increased overall survival in patients who had already received two previous chemotherapy rounds, but appeared to be detrimental in those who already received three chemotherapy rounds.
Giovanni Scambia, Ricardo Villalobos Valencia, Nicoletta Colombo, David Cibula, Charles A Leath 3rd, Mariusz Bidziński, Jae-Weon Kim, Joo Hyun Nam, Radoslaw Madry, Carlos Hernández, Paulo A R Mora, Sang Young Ryu, Mei-Lin Ah-See, Elizabeth S Lowe, Natalia Lukashchuk, Dave Carter, Richard T Penson.
J Clin Oncol 2024. PMID: 39668137.

Microwave- vs radiofrequency- based renal tumor ablation. Through the retrospective analysis of over 500 early stage renal cancer patients who underwent tumor ablation by either microwave or radiofrequency, the authors showed a greater efficacy of microwave-based tumor ablation, with complete tumor removal, no severe complications, no reduction in renal functioning. Moreover, the operative time of microwave-based tumor ablation was shorter.
Letizia Maria Ippolita Jannello, Franco Orsi, Stefano Luzzago, Giovanni Mauri, Francesco A Mistretta, Mattia Luca Piccinelli, Chiara Vaccaro, Marco Tozzi, Daniele Maiettini, Gianluca Varano, Stefano Caramella, Paolo Della Vigna, Matteo Ferro, Guido Bonomo, Zhe Tian, Pierre I Karakiewicz, Ottavio De Cobelli, Gennaro Musi.
BJU Int 2025. PMID: 39290073.

Methodological factors influence the predictive power of MRI-radiomics in estimating response to neoadjuvant chemotherapy. Through a systematic review, the authors showed that despite the potential of MRI-radiomics in predicting response to neoadjuvant treatment of breast cancer patients, methods for

image acquisition strongly affect the accuracy of the predictive models; therefore, standardized protocols are urgently needed to fully exploit the potential of such tools.
Sofia Netti, Oriana D'Ecclesiis, Federica Corso, Francesca Botta, Daniela Origgi, Filippo Pesapane, Giorgio Maria Agazzi, Anna Rotili, Aurora Gaeta, Elisa Scalco, Giovanna Rizzo, Barbara Alicja Jereczek-Fossa, Enrico Cassano, Giuseppe Curigliano, Sara Gandini, Sara Raimondi.
Eur Radiol 2024. PMID: 39702630.

Human brain organoids in neurodevelopmental research - two new experimental approaches. Human neurodevelopmental studies strongly rely on the use of brain organoids and single cell-resolved multi-omic technological approaches. However, while representing an excellent disease model, scaling up from the analysis of a single organoid to a whole patient cohort is still limited by technical issues; yet, it would allow to unveil the molecular causes of neurodevelopmental disorders. The authors describe the set-up of two new approaches for human brain organoid multiplexing (that is, pooling together different cell populations for experimental purposes and then deconvolve -demultiplexing- the cellular heterogeneity of the cell pool by single cell-resolved analyses). One approach, best suited for large scale analyses, entails the pooling of patient-derived (pluripotent) cells for the generation of a "mosaic" organoid (containing cells from the different donors); another approach, instead, foresees the pooling of donor-derived organoids (after dissociation into single cell suspensions). Then, both strategies employ a computational tool -SCanSNP- enabling (genotype-based) single cell type identification (within the organoid).
Nicolò Caporale #, Davide Castaldi #, Marco Tullio Rigoli #, Cristina Cheroni, Alessia Valenti, Sarah Stucchi, Manuel Lessi, Davide Bulgheresi, Sebastiano Trattaro, Martina Pezzali, Alessandro Vitriolo, Alejandro Lopez-Tobon, Matteo Bonfanti, Dario Ricca, Katharina T Schmid, Matthias Heinig, Fabian J Theis, Carlo Emanuele Villa, Giuseppe Testa.
Nat Methods 2024. PMID: 39653820.

Guidelines for the exploitation of human organoids in research. In this review, the authors provide guidelines for the employment of brain organoids in research, covering aspects such as pluripotent cell quality, characterization of derived cells, cell manipulation, in-mice transplantation, to ensure high quality research results and accelerate our understanding of human neurodevelopment, in physiological and disease contexts.
Sergiu P Pașca, Paola Arlotta, Helen S Bateup, J Gray Camp, Silvia Cappello, Fred H Gage, Jürgen A Knoblich, Arnold R Kriegstein, Madeline A Lancaster, Guo-Li Ming, Gaia Novarino, Hideyuki Okano, Malin Parmar, In-Hyun Park, Orly Reiner, Hongjun Song, Lorenz Studer, Jun Takahashi, Sally Temple, Giuseppe Testa, Barbara Treutlein, Flora M Vaccarino, Pierre Vanderhaeghen, Tracy Young-Pearse.
Nature 2024. PMID: 39653126.

Metabolomics and lipoproteomics to distinguish celiac disease and non-celiac gluten sensitivity. The authors found that blood metabolites (mainly HDL cholesterol-related) and lipoproteins-linked parameters can distinguish celiac individuals from those with non-celiac gluten sensitivity (which does not involve allergic or autoimmune mechanisms). Due to the known effect of the gut microbiota on cholesterol homeostasis and blood lipid composition, these data suggest the existence, in these individuals, of gut microbiota dysbiosis.
Alessia Vignoli, Claudio Luchinat, Nicola Segata, Daniela Renzi, Leonardo Tenori, Antonino Salvatore Calabrò.
Clin Nutr 2024. PMID: 39736173.

Genomics and mass spectrometry for the monitoring of antibiotic resistance. By combining genomic (whole genome sequencing and variant calling) and functional (mass spectrometry-based detection and quantification of the intracellularly accumulated antibiotic in sensitive vs resistant bacteria) analyses, the authors identified a novel clinically relevant mutation of *Acinetobacter baumannii* (a bacterium known for causing human infections) responsible for the bacterial resistance to Cefiderocol (by reducing antibiotic uptake and intracellular accumulation), and demonstrated the usefulness of this approach for the improved monitoring of the emergence of new antimicrobial resistance-related mutations in a routine setting.

Lavinia Morosi #, Davide Golzato #, Linda Bussini, Hygerda Guma, Federica Tordato, Federica Armanini, Zian Asif, Francesco Carella, Paola Morelli, Michele Bartoletti, Giorgio Da Rin, Erminia Casari, Giuseppe Martano, Maria Rescigno, Nicola Segata, Sara Carloni, Valeria Cento.
Front Microbiol 2024. PMID: 39744402.

What do clinicians think about the use of social media for the physicians-patient interaction? In this study the authors performed a survey to understand how clinicians feel about the use of social media in their interaction with patients. On the basis of a survey including 116 participants, the authors identified three different social media-related clinician profiles: The frequent user, the intermediate user and the skeptical user (31, 38, 31% respectively). Notably, greater consideration of the use of social media for professional purposes was associated with older age, overall time spent on social media, and specialization level.
Elena Battaiotto, Carmine Valenza, Mattia Garutti, Luigi Orlando Molendini, Elena Bellio, Dario Trapani, Fabio Puglisi, Gabriella Pravettoni, Luca Buccoliero, Giuseppe Curigliano, Manuelita Mazza.
JCO Glob Oncol 2025. PMID: 39752614.

What else is new in science?

Metabolomic analyses reveal the metabolite recapitulating caloric restriction effects. Increasing scientific evidence indicate positive effects of caloric restriction (CR) on human health. Preclinical studies also proved its efficacy as an anticancer tool. In this work, the authors show, in in vivo preclinical models, the CR-induced increase of the lithocholic acid (LCA) metabolite in CR-fed mice. Importantly, LCA administration –at concentrations below those causing DNA damage or tumor development– is sufficient to recapitulate the positive effects of caloric restriction.
Nature 2024. PMID: 39695227.

Fructose limits tumor growth by non cell-autonomous mechanisms. The authors found that, in preclinical in vivo models, fructose administration increased tumor growth, whereas in vivo inhibition of the enzyme involved in fructose lysis (ketohexokinase-C) –expressed by hepatocytes but not by cancer cells–, while not affecting directly cancer cells, prevented fructose-dependent tumor growth, indicating the existence of a non-cell autonomous mechanism.
Nature 2024. PMID: 39633044.

The metabolic network influencing tumor cells and tumor microenvironment. In this review, the authors delve into the effects of metabolic reprogramming of tumor cells and tumor microenvironment, as well as the alteration of the tumor-microenvironment interaction, on tumor progression. They also discuss the potential of metabolism-targeted therapeutic approaches as potential new and effective strategies.
Mol Cancer 2025. PMID: 39789606.

Cancer and metabolism - a review. In this review the authors cover a number of aspects related to cancer-induced metabolism reprogramming, including the metabolic changes at the level of the single cancer cells, of the tumor microenvironment, as well as the systemic changes. Interaction and mutual influence of all these factors contribute to cancer onset, progression and response to therapy. A deep understanding of such mechanisms is critical for designing novel and effective therapeutic approaches.
Nat Rev Cancer 2025. PMID: 39833533.

Nutrient metabolism and T cell exhaustion through epigenetic reprogramming. Exhausted T cells are less active immune cells with reduced antitumor ability. T cell exhaustion has been shown to be related to metabolic and epigenetic reprogramming. The authors describe the metabolic shifts T cells undergo when becoming exhausted (from acetate to citrate metabolism, through the downregulation of acetyl-CoA synthetase 2, ACSS2) resulting in the altered epigenetic profile of cells (such as KAT2A-ACLY-mediated

increased histone acetylation). Overexpression of ACSS2 or inhibition of ACLY are able to prevent T cell exhaustion.
Science 2024. PMID: 39666821.

Targeting the microglia to enhance antitumor immunity against melanoma brain metastases. The authors unveil a mechanism through which microglia cells promote melanoma metastases in the brain, through the activation of NF-kB pathway. Indeed, inhibition of this pathway induces microglia reprogramming toward a proinflammatory phenotype, resulting in increased antitumor immunity and reduced brain metastases. Consistently, in patients, microglia markers of inflammation (within melanoma brain metastases) correlate with greater response to immunotherapy (immune checkpoint inhibitors) and, in preclinical in vivo models, the co-administration of immunotherapy and NFkB pathway blockers improved therapy response.
Cancer Cell 2025. PMID: 39919736.

The dual role of neurons in cancer: Multifaceted aspects of cancer-neuroscience. Neurons-tumors proximity has several consequences: On one side, neurons can promote cell proliferation and angiogenesis, and influence the immune cells, fostering tumor progression; on the other side, neuronal activity may have a suppressive effect on tumorigenesis. In this review, the authors collect the current knowledge regarding this tumor-neurons interaction, whose deeper understanding may allow for its targeting with therapeutic scopes.
Mol Cancer 2025. PMID: 39915765.

Brain metastases - a disease of the nervous system? In this review, the authors discuss the concept of considering brain metastases as a disease of the central nervous system rather than strictly correlated to the tumor of origin, due to the profound changes cancer cells undergo to disseminate to the brain, survive and grow, suggesting that this change of mind may allow to rationally develop more efficient therapeutic approaches.
Lancet Oncology 2025. PMID: 39914421.

Cancer cell-intrinsic electrical activity and tumorigenesis. Small cell lung cancer is a highly heterogeneous tumor type, and contains both cancer cells with neuroendocrine features (NE) and cells without neuroendocrine features (non-NE). Through the electrophysiological characterization of lung cancer cell lines and patient-derived xenografts, the authors showed that electrical activity of NE lung cancer cells directly induces tumorigenesis and supports cancer cell metastatic potential, although at the same time creating a metabolic ATP-linked vulnerability (increased OXPHOS dependency) of these cancer cells, which need high levels of ATP to sustain their electrical activity. Moreover, non-NE lung cancer cells interact with NE cancer cells by providing metabolic support to sustain NE cancer cells' electrical activity.
Nature 2025. PMID: 39939778.

Long non-coding RNAs in cancer adaptation. During cancer progression, cancer cells face harsh and diverse microenvironmental conditions and, to survive, they need to adapt. Cancer cell adaptation entails a number of different mechanisms. Protein synthesis is often altered in cancer and lncRNAs play a key role in modulating protein synthesis during cancer progression and adaptation, for instance, by contributing to regulate metabolic adaptation to stress and maintaining high levels of oncogene expression, they sustain cancer progression. In this review, the authors delve into the role of lncRNAs in cancer plasticity and resistance to stress.
Mol Cancer 2025. PMID: 39891197.

Overall survival of pembrolizumab therapy in early stage triple negative breast cancer patients. In the frame of the phase III KEYNOTE-522 trial, which already showed improved pathological complete response and event-free survival in early stage triple negative breast cancer patients treated with pembrolizumab and platinum-containing chemotherapy in the neoadjuvant setting followed by

pembrolizumab in the adjuvant setting, the authors found a significant improvement in overall survival as compared to neoadjuvant chemotherapy alone.
N Engl J Med 2024. PMID: 39282906.

Neoadjuvant pembrolizumab for the treatment of high risk, early stage ER+/HER2- breast cancer. In the frame of a phase III clinical trial, the authors showed that in high risk, previously untreated, early stage ER+/HER2- breast cancer patients, the addition of pembrolizumab to chemotherapy, in the neoadjuvant setting, increased pathological complete response (as compared to placebo-chemotherapy treatment), with comparable adverse events.
Nat Med 2025. PMID: 39838117.

Is radiotherapy-chemoradiotherapy-immunotherapy combination an option for the treatment of lung cancer? In the frame of a phase II trial, the authors tested the feasibility of a therapeutic approach combining radiotherapy (tumour stereotactic body radiotherapy, SBRT) followed by chemoradiotherapy to the lymph nodes, and consolidation immunotherapy in non-resectable, locally advanced lung cancer patients. Results indicate a favorable safety and efficacy profile of this combined approach as compared to other chemoradiotherapy-based approaches, and served as a basis for the launch of anongoing phase III trial.
Lancet Oncol 2025. PMID: 39615497.

The role of the immune system in metastatic colonization. In this review, the authors discuss the current knowledge regarding the role of the immune system in shaping the pre-metastatic and metastatic niche in the liver, supporting metastasis formation, as well as the therapeutic tools currently being explored aimed at interfering with the immune niche to counter the metastatic disease.
Mol Cancer 2024. PMID: 39543660.

The MESSAGE project on sex and gender in medical research. In this comment, the authors summarize the work of the Medical Science Sex and Gender Equity *(MESSAGE)* project for the establishment of pragmatic gold standard for sex and gender inclusion in research design.
Lancet 2024. PMID: 39541996.

Sex and gender in colorectal cancer (CRC) progression. Sex-related differences in CRC-related mortality are known. In this paper, by leveraging in vitro and in vivo models of CRC, the authors analyzed how sex and gender influenced the interaction between the immune system and the gut microbiome in CRC progression. Their results revealed differences in female vs male CRC patients in terms of immune cell subtypes infiltrating the tumor mass and enrichment of gut microbiome species. The female patients-specific gut microbiota composition had an effect on immune cells, reducing the antitumor activity of iNKT cells. Therefore, the authors provided a mechanistic explanation of the observed sex-related differences in CRC patients, which may be clinically relevant in a therapeutic setting.
Oncoimmunology 2024. PMID: 39548749.

Early onset colorectal cancer (CRC) incidence: an analysis. In this work the authors analyzed and compared the trend in the incidence of both early onset (<50yrs) and late onset (>50yrs) CRC, in different countries in the world, by exploiting data extracted from the WHO-International Agency for Research on Cancer Cancer Incidence in Five Continents Plus database. They found that early onset CRC is increasingly detected in about half (27 of 50) of the countries analyzed, being more frequently detected than late onset CRC; that is, the rise in the number of cases detected appeared steeper for early onset than for late onset CRC, encouraging studies aimed at analyzing the underlying reasons and promoting approaches for early detection.
Lancet Oncol 2025. PMID: 39674189.

Cancer-associated fibroblasts (CAFs) and colorectal cancer growth. The authors showed a mechanism through which a specific subset of CAFs (the THBS2+ CAFs) induces epithelial-to-mesenchymal transition (EMT) and promotes cancer cell resistance to the chemotherapeutic oxaliplatin, through the secretion of COL8A1 and the ensuing binging to ITGB1 expressed on oxaliplatin-resistant cancer cells. Consistently, inhibition of COL8A1 limits cancer progression and increases cancer cell sensitivity to oxaliplatin.
Mol Cancer 2024. PMID: 39732719.

How to get a mechanistic view of the gut microbiota - a review. In this review, the authors discuss the conventional and innovative chemical and genetic approaches to dissect mechanisms and pathways linked to different gut microbiota species in health, disease and therapy, to reveal causal relations and foster the broad employment of microbiota-focused therapeutic interventions.
Science 2024. PMID: 39541443.

Strain richness of the gut microbiota. The authors determined the strain richness (that is, the number of different bacterial breeds/strains) in the gut, highlighting that it is species-specific and can be temporarily modified by therapeutic manipulation. Engraftment of administered strains correlates with the strain richness prior to treatment, which may affect outcome of fecal microbiota transplantation and therapeutic probiotic administration.
Nature 2025. PMID: 39604726.

Gut microbiome analysis of african populations. A large body of scientific studies demonstrated that the gut microbiome is influenced by a number of aspects including geographical, genetic, environmental, lifestyle-related factors. The authors report the results of the "AWI-Gen 2 Microbiome Project", sampling the microbiome of 1801 women from different african countries, encompassing a wide range of different conditions from rural to urban settings, thus providing useful data for future gut microbiomes studies.
Nature 2025. PMID: 39880958.

Oral microbiota dysbiosis promotes head and neck cancer (HNC). The authors showed that, in preclinical in vivo models, oral microbiota dysbiosis (characterised by increased abundance of Pseudomonas and Veillonella) induced by chronic stress directly promotes HNC onset. Moreover, oral microbiota dysbiosis modifies the plasma metabolome, increasing the levels of kynurenine (Kyn). The higher Kyn levels promote T cell exhaustion, through AhR (Kyn receptor) deubiquitination, and HNC tumorigenesis.
Gut 2025. PMID: 39904603.

Mechanisms of double-strand break (DSB) repair. The authors describe the mechanisms through which cohesin oligomers tether DSB ends, promoting DNA repair, thus revealing a role for cohesin in safeguarding genome integrity.
Nat Cell Biol 2025. PMID: 39482358.

Glucocorticoid receptor, GATA6 and RNA Pol II in the modulation of pancreatic cancer (PDAC) subtypes. Glucocorticoid agonists induce glucocorticoid receptor interaction with the transcription factor GATA6, in turn modulating Pol II pause release and the transcriptional program underlying the classical (as opposed to basal) PDAC subtype: Upon treatment with glucocorticoid agonists, by facilitating Pol II pause release, GATA6 ends up inhibiting the classical PDAC subtype. By revealing key molecular mechanisms underlying the classical PDAC subtype establishment, the authors unveil a new potentially actionable mechanism exploitable in a therapeutic setting and emphasize that glucocorticoid receptor agonists, employed in the clinics, may actually promote a more aggressive tumor behavior.
Gut 2025. PMID: 39884837.

Histone gene hyper-transcription and tumor aggressiveness. By comparing RNA pol activity in normal and tumoral tissues, the authors showed that high RNA occupancy at histone genes is parallel to high

tumor aggressiveness, tumor recurrence and chromosome losses, and suggest that hyper-transcription of histone genes may represent the mechanistic driver of cancer hyper-proliferation.
Science 2025. PMID: 39946483.

In the tumor microenvironment, markers of response to immunotherapy of gastric cancer patients with peritoneal metastases. In the frame of a phase II clinical trial, through the single-cell profiling of patients-derived primary tumors, metastases, and peripheral blood, the authors revealed the features of the tumor microenvironment associated with resistance to immune checkpoint inhibitor therapy, which may help in selecting patients likely to respond to immunotherapy.
Gut 2024. PMID: 39537239.

Mechanisms of immune evasion of tumor initiating cells (TICs). The authors found that, in hepatocellular carcinoma, CD49f protein is a marker of TICs. Moreover, TICs expressing high levels of CD49f are able to recruit tumor-promoting neutrophils and create an immunosuppressive environment that fosters cancer progression. CD49f-mediated alteration/stabilization of CD155 expression TIC immune evasion and CD155 blockade or deletion increases sensitivity to anti-PD1 immunotherapy, revealing a new targetable mechanism of tumor immune evasion.
Cancer Cell 2024. PMID: 39515328.

In a review, the genetic and epigenetic features associated with long-term, adverse effects of cancer therapy in childhood cancer patients. Survivors of pediatric cancer frequently display adverse events related to therapy, such as different chronic conditions, as well as secondary neoplasms. In this review, the authors present the current knowledge regarding the molecular -genetic and epigenetic- effects of therapy in childhood cancer survivors, with main focus on secondary neoplasms and cardio-myopathies.
Nat Rev Cancer 2024. PMID: 39511414.

Single cell-resolved, spatial characterization of breast cancer microenvironment. The authors performed the integrated analysis of single cell and single nucleus RNAseq, spatial expression assays, Hematoxylin & Eosin staining of metastatic breast cancer patient biopsies, from different anatomical sites, and in different clinico-pathological situations, to describe the tumor microenvironment, highlighting clinically relevant traits.
Nat Med 2024. PMID: 39478111.

Unveiling the mechanisms of resistance of lung cancer brain metastases to tyrosine kinase inhibitors (TKIs). Through single cell RNAseq analyses of patient-derived tumor specimens, the authors unraveled key mechanisms of resistance of lung cancer brain metastases to TKIs, which involve the increased expression of the CTLA4 immune checkpoint in T cells and the ensuing generation of an immunosuppressive environment. In in vivo murine models, administering CTLA4 blockers along with TKIs results in enhanced therapy efficacy.
Cancer Cell 2024. PMID: 39423817.

Insights into the link between aging and cancer. Two papers recently published *Science* and *Nature* unraveled critical cell-intrinsic and cell-extrinsic factors influencing tumorigenesis. On one hand, by leveraging preclinical in vivo models, the authors demonstrated the pivotal role of immune system age in lung cancer progression, showing that, independently from the age of the stroma, aging of the immune system was associated with cancer growth. Moreover, in in vivo preclinical models, reconstituting old mice with a youthful immune system was able to rescue their antitumor immune activity and delay lung cancer progression. They found that IL1 played a major role in promoting immunosuppression and, consistently, inhibiting IL1 activity slowed down tumor progression. On the other hand, along with the cell-extrinsic (that is, environmental, immune system-related) effects of aging on tumor development, the authors of another study showed a cell-intrinsic effect of aging on lung cancer progression, revealing that aging led to a change in DNA methylation, inducing the expression of Nupr1 protein, which in turn

resulted in iron dysregulation in stem cells, ultimately limiting the self-renewal capacity of stem cells and, as a consequence, reducing tumor initiation.
Science 2024. PMID: 39236155; Nature 2024. PMID: 39633048.

Exploiting intratumor bacteria as cancer vaccines. By exploiting the natural homing of some bacteria towards tumors, the authors developed an bacterial antitumor vaccine platform based on the engineered probiotic Escherichia coli Nissle 1917, producing peptides fostering immune system-mediated clearance of tumor cells, resulting in a potent and specific T cell-mediated anticancer immunity that effectively controls or eliminates tumour growth and extends survival in advanced murine primary and metastatic solid tumours.
Nature 2024. PMID: 39415001.

The role of tissue-resident macrophages in dormancy of breast cancer metastases. The mechanisms underlying long-term dormancy of disseminated cancer cells, as well as the ensuing awakening and fueling of metastasis growth, are being intensely investigated. In this paper, the authors found that TGFbeta2 -expressed on alveolar macrophages- interaction with its receptor -on cancer cells- plays a key role in maintaining breast cancer cell dormancy in the lungs for extended periods. The loss of this interaction results in metastatic awakening, demonstrating that TGFbeta2-expressing macrophages act as "switches" of breast cancer metastatic colonization in the lung.
Cell 2024. PMID: 39378878.

Cell plasticity of metastatic colorectal cancer cells. By comparing matched normal, primary and metastatic colorectal cancer (CRC) patient samples, the authors unveiled the high plasticity of CRC metastases, which progressively lose intestinal cell identity, first becoming undifferentiated (reprogramming into a fetal progenitor state) and then acquiring squamous and neuroendocrine traits. This process of dedifferentiation-redifferentiation is increased in metastases, is fostered by chemotherapy, and correlates with poor patient survival. Moreover, in in vitro 3D cultures, metastatic cells display greater cell-autonomous ability to differentiate in different cell lineages in response to microenvironmental stimuli as compared to the primary tumors. PROX1 protein plays a key role in this process.
Nature 2025. PMID: 39478232.

Focus on early onset colorectal cancer (CRC). In this review, the authors discuss the the current lack of solid information on the increasingly frequent early onset CRC, hypothesizing a greater aggressive behavior and "fast" evolution of the early onset CRC as compared to the standard onset CRC, and highlighting the challenges to be faced to obtain reliable predictions on tumor behavior; namely, the timing of tumor progression/evolution, to estimate tumor "age" (that is, time from initial neoplastic transformation to clinical detection), a clinically relevant aspect to design adequate screening campaigns.
Cell 2025. PMID: 39919707.

Features identifying colorectal cancers (CRC) responding to immune checkpoint inhibitors (ICI). The authors showed that ICI efficacy, both in mismatch repair deficient (dMMR) and in a subset of mismatch repair proficient (pMMR) CRCs, relies on the presence of IFN-secreting cytotoxic T lymphocytes and CD74-expressing antigen-presenting macrophages in the tumor microenvironment, as well as CD74 expression by tumor cells. Moreover, some patients with pMMR displayed better progression-free survival upon treatment with ICI.
Cancer Cell 2025. PMID: 39824178.

***Ex vivo* white blood cell manipulation-induced increase of adiponectin reduces immune-related adverse events**. Anti-PD1 and anti-CTLA4 immunotherapy have shown remarkable results in several tumor types. However, the beneficial effects of this therapeutic approach are variable, and treatment administration is often accompanied by immunotherapy-related adverse events that can lead to

treatment discontinuation. In this study the authors showed, both in preclinical in vivo models and in the frame of a phase1b/2 clinical trial, that through the induction of adiponectin expression, extracorporeal photopheresis (ECP, ex vivo white blood cell manipulation, in which cytotoxic T cells from the patient's blood are depleted outside of the body before the blood is re-infused, in order to downregulated pathogenic T cell activity) reduced the number of pathogenic immune cells, thus decreasing inflammation, and ultimately significantly limiting immune-related adverse events in immunotherapy-treated patients, without impairing antitumor immunity.
Cancer Cell 2025. PMID: 39933899.

Mechanisms of Sorafenib resistance. The authors revealed a microenvironmental mechanism contributing to liver cancer resistance to Sorafeninb, an FDA-approved drug for advanced stage liver cancer that frequently results in drug resistance. Their results demonstrate that sorafenib treatment promotes an immune suppressive environment, by increasing the (CCR2-mediated) recruitment of myeloid-derived suppressor cells (MDSCs) inside the tumor and enhancing their activity, through the (PPARalpha-mediated) modulation of fatty acid oxidation, ultimately supporting tumor growth. Moreover, tumor-bearing mice under treatment with sorafenib and fed a high fat diet show a worse outcome as compared to mice fed a regular diet.
Mol Cancer 2025. PMID: 39876004.

The prognostic power of ctDNA analysis-based minimal residual disease - results of the CIRCULATE-Japan GALAXY observational study. The authors reported the final analysis results of the CIRCULATE-Japan GALAXY observational study, which has previously shown the association between minimal residual disease (MRD) -as detected by ctDNA analysis- and risk of recurrence, and the benefits of adjuvant therapy in colorectal cancer patients. In this work, they showed that at 23-month median followup, colorectal cancer patients at stage II, III, IV, ctDNA detection correlates with shorter disease-free survival and overall survival. In relapsing patients, sustained clearance of ctDNA after adjuvant chemotherapy was associated with better survival as compared to transient ctDNA clearance, strongly supporting the use of ctDNA analysis in patient stratification, to inform on decision making regarding the adjuvant treatment.
Nat Med 2024. PMID: 39284954.

Extracellular vesicles (EVs) in the modulation of cancer immunity. In this review, the authors provide an overview on the current knowledge regarding the role of EVs in modulating the antitumor activity of the immune system, and their role in anticancer therapy.
Mol Cancer 2025. PMID: 39953480.

Aneuploid cells in healthy mammary tissue. The authors showed that in healthy human mammary tissue there are rare aneuploid cells –some of them characterized by specific chromosomal aberrations typical of invasive breast cancers– whose number increases with age and mainly localized in morphologically healthy ductal and lobular structures.
Nature 2024. PMID: 39567687.

Mechanisms of chromosome instability (CIN). Chromosome instability is a frequent feature of solid tumors. The authors found that, in lung cancer, genomic alterations of FAT1 gene impair homologous recombination (HR); FAT1 loss causes -via YAP1- cell stress and CIN.
Nat Cell Biol 2024. PMID: 39738653.

Chromothripsis - a review. Chromothripsis -namely the deep rearrangements of one or few chromosomes- is frequently found in cancer. The authors discuss the current knowledge regarding initiation and consequences of chromothripsis, its main features and contribution to cancer development, and the potential of its exploitation in a therapeutic perspective, as a targetable cancer vulnerability.
Nat Rev Cancer 2024. PMID: 39548283.

HNOCA - A transcriptomic atlas of human neural organoids. In this paper, the authors reported the development of a single cell RNA seq-based atlas of neural organoids of human origin, by integrating data from 36 datasets, for a total of 1.77 million cells, providing the scientific community with a useful source for studying molecular aspect of diseases. The tool is freely available through a user-friendly web-based interface.
Nature 2024. PMID: 39567792.

Neutrophils in brain tumors and brain metastases. By comparing tumor-associated neutrophils from human brain tumors and brain metastases with circulating neutrophils, the authors showed that brain tumor-associated neutrophils have a largely different profile, characterized by immune-suppressive capacity.
Cell 2023. PMID: 37769657.

Polycomb complex and epigenetic inheritance. The authors defined the mechanism underlying the re-establishment of histone H2A lysine 119 monoubiquitination (H2AK119Ub), by the non-canonical PRC1-containing RYBP, after DNA replication.
Nature 2024. PMID: 39537923.

A new approach for the parallel genetic and epigenetic sequencing. In this paper, the authors report the set-up of a methodology enabling, with low DNA input, the parallel, accurate sequencing, at single base resolution, of DNA sequence as well as two main epigenetic DNA modifications (the two most common cytosine modifications). The approach was validated by using both human genomic DNA and circulating free DNA.
Nat Biotechnol 2023. PMID: 36747096.

Single cell and spatial transcriptomics - the state of the art. In this review the authors discuss latest advances and future challenges in the exploitation of single cell and spatial transcriptomics in research, two valuable tools to describe, with high resolution, cell phenotype and cell interaction with their microenvironment. They also examine the increasing combination of these technologies with machine learning tools, their current use in immunology, stem cell and cancer biology, and their potential future application in the clinical setting.
Nat Rev Mol Cell Biol 2025. PMID: 39169166.

KRAS mutations in cancer: Cell-intrinsic and microenvironmental aspects. KRAS mutations are frequent in cancer and their oncogenic role makes KRAS an interesting pharmacological target. In these two reviews, the authors provide an overview of the current knowledge regarding the mechanisms related to mutated KRAS-induced alterations of cancer cell metabolism and epigenetic profile, as well as the effect on the tumor microenvironment. Representing potentially effective therapeutic targets in KRAS-driven cancers, the authors discuss the currently available therapeutic strategies targeting such mechanisms.
Mol Cancer 2025. PMID: 39806421 and 39799325.

MSK-CHORD: a clinico-genomic real world cancer dataset. By combining natural language processing of clinical annotations with structured data (such as those regarding medication, demographics, tumor genomic), the authors generated a real world dataset (MSK-CHORD) of clinical and genomic data, including almost 25.000 patients with lung, breast, colorectal pancreatic cancers, treated at the Memorial Sloan Kettering Cancer Center, providing a useful tool for cancer researchers. Machine learning models trained by leveraging this huge dataset and predicting overall survival showed that data derived from unstructured information (such as information on the disease site) outperform those trained only on genomic data or tumor stage.
Nature 2024. PMID: 39506116.

Genomic profiling of early stage bladder cancer. The authors report the genomic description of early stage (non-muscle invasive) bladder cancer, based on whole-exome sequencing, shallow whole-genome sequencing, RNA sequencing analysis, from 438 patients, in relation to disease aggressiveness. This study highlights the high genomic variation of this tumor type. Moreover, tumors with high copy numbers correlated with worse patient outcomes.
Nat Genet 2025. PMID: 39753772.

Mutations conferring resistance to immune checkpoint inhibitors (ICI) in melanoma. Through the comparative genomic analysis of melanoma biopsies before ICI therapy and after acquiring treatment resistance, the authors identified some resistance-associated mutations, such as those influencing interferon signaling: Mutations in SEC24 gene resulted in impaired (IFN-regulating) STING signaling and lower T cell activation.
Cancer Cell 2025. PMID: 39933900.

The state of the art on the use of artificial intelligence (AI) in healthcare. In this review the authors offer an overview on the state of the art, challenges and future perspectives regarding the use of AI-based tools for integrating patient information into multi-modal algorithms that can ultimately aid clinicians in the diagnostic and prognostic setting.
Lancet Oncol 2024. PMID: 39637906.

Generalizing results from controlled clinical trials in a real world setting. By leveraging a database of electronic medical records, the authors developed "TrialTranslator", a machine learning-based model to assess whether results collected in the frame of controlled oncological clinical trials –in which patients are selected on the basis of sometimes very restrictive criteria– hold true when applied in a real world setting. Their results show that for low- and medium- risk patients, results, in terms of survival, usually coincide, whereas they significantly diverge in high-risk patients (in which survival is usually lower in real world scenarios). TraiTranslator represents a useful tool to aid clinicians for making informed treatment decisions, allowing them to weigh the benefits of a new therapy.
Nat Med 2025. PMID: 39753967.

In a review, the state of the art on the relation between hematopoietic stem cells (HSC) and the developmental niche. The authors discuss the current knowledge regarding the interaction between HSC and the niche, and the microenvironmental signals that, during development and aging, non-cell autonomously modulate HSC maturation. In-depth investigation of such mechanistic interactions may reveal useful disease-related and therapeutically relevant information.
Nat Rev Mol Cell Biol 2025. PMID: 39256623.

GDF-15 blockade to increase anti-PD1 immunotherapy efficacy – results of a first phase 1-2a clinical trial. In this work, the authors report the results of the first clinical study showing the effect of the inhibition of GDF-15 cytokine (by means of the neutralizing antibody visugromab) in combination with the anti-PD1 antibody nivolumab in patients with advanced solid tumors refractory to anti-PD1 immunotherapy. The combined administration of visugromab and nivolumab induced durable response in some patients, holding promises to overcome resistance to immune checkpoint inhibitors.
Nature 2024. PMID: 39663448.

IL-4 and ovarian cancer resistance to immunotherapy. Despite the remarkable effects of immunotherapy in the treatment of several tumor types, its clinical efficacy is limited in ovarian cancer patients, due to both intratumor heterogeneity and an immunosuppressive microenvironment. The authors show that ovarian cancer cells-secreted IL4 is one of the main causes of patient resistance: By acting on the macrophages, IL4 induces a strongly immunosuppressive microenvironment, and its targeting may represent a putative strategy to increase patient response to immunotherapy.
Cell 2024. PMID: 39481380.

A machine learning model for ultrasound-based ovarian cancer diagnosis. By using a dataset of over 17.000 ultrasound images from over 3.000 ovarian cancer patients, in 20 different care centers, the authors developed a deep learning tool for ovarian cancer detection, with excellent diagnostic performance even in highly heterogeneous technical conditions (such as different ultrasound systems, patient subgroups, and histological diagnoses).
Nat Med 2025. PMID: 39747679.

DIAMO IL BENVENUTO A

WE WELCOME

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Cover image *(by Giuliana Pelicci, Massimiliano Del Bene):*

Transmission electron micrograph of GBM tissue (green) releasing vesicles in the extracellular space. Exosomes are shown by white arrows, whereas microvesicles and large oncosomes are shown by black arrows.

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