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...in Lab



Modelli preclinici di neuroblastoma per la valutazione degli effetti antitumorali di strategie terapeutiche di medicina personalizzata e di precisione

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IRCCS Istituto G. Gaslini, Genova*

Milano, 22 e 23 maggio 2026

Nulla da dichiarare

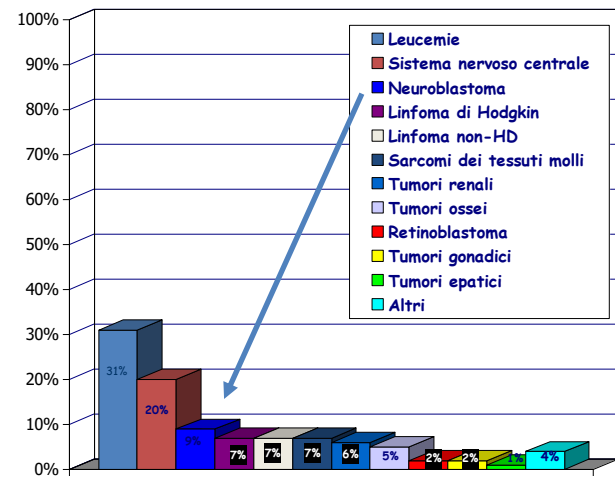
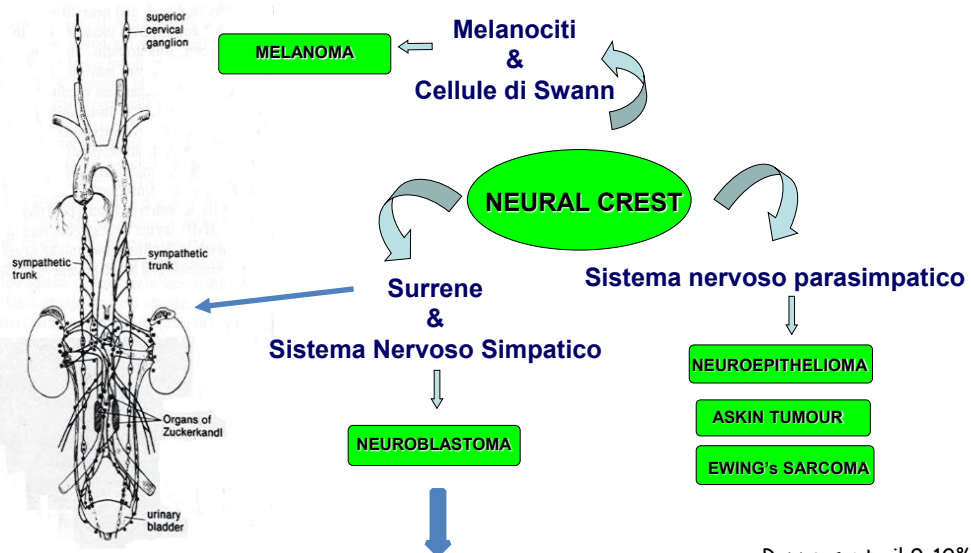
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Neuroblastoma

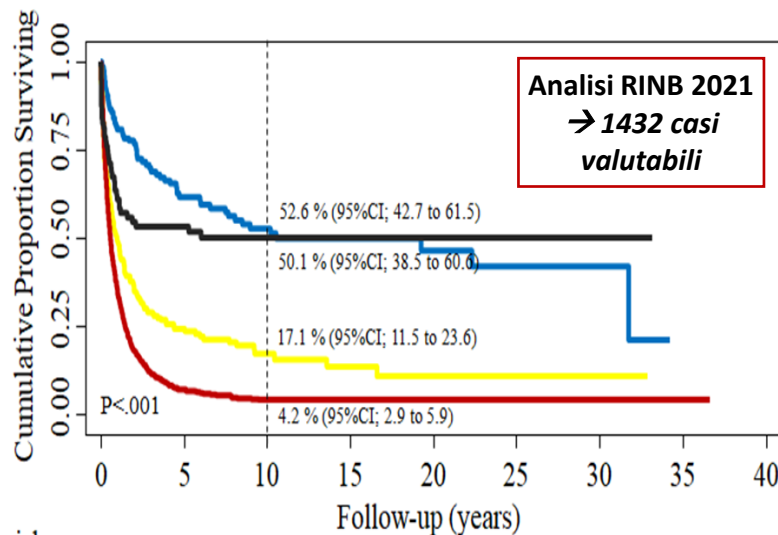


Localizzazione del SNS
(gangli e midollare del surrene)

Rappresenta il 9-10% dei tumori pediatrici
(~ 125 nuovi casi/anno in Italia, > 10.000 nel mondo)

- origine neuroectodermica
- deriva dalle cellule della cresta neurale
- il più comune tumore solido extracranico e la più comune causa di morte per malattia in età pre-scolare
- il più comune tumore congenito (su 10 neonati con tumore almeno 6 sono affetti da neuroblastoma)

Neuroblastoma alto rischio (stadio M > 18m) in recidiva



Outcome di pazienti affetti da NB alto rischio, e soprattutto di quelli refrattari- recidivi rimane inaccettabilmente

Nuovi approcci terapeutici

Modelli clinicamente rilevanti

Number at risk

	0	5	10	15	20	25	30	35	40
Stage L1	126	64	40	22	14	5	4	0	0
Stage L2	210	34	11	6	2	2	1	0	0
Stage M	1009	50	20	7	5	2	1	1	1
Stage Ms	80	36	25	16	11	3	1	0	0

Ricerca traslazionale nel NB: rilevanza clinica modelli animali

Cellule **murine** in topo immunocompetente (s.c.)

modello sottocute (proof-of-concept)

Non rispecchia la clinica

vs modelli clinicamente rilevanti
microambiente tumorale «limitato»

- infiltrato linfocitario inferiore
- risposta alle immunoterapie inferiore
- endotelio tumorale ridotto

Cellule **umane** in topo immunodepresso (s.c.)

Cellule **umane** in topo immunodepresso (i.v.)
(pseudo)metastatico

Cellule **umane** in topo immunodepresso (surrene) ortotopico

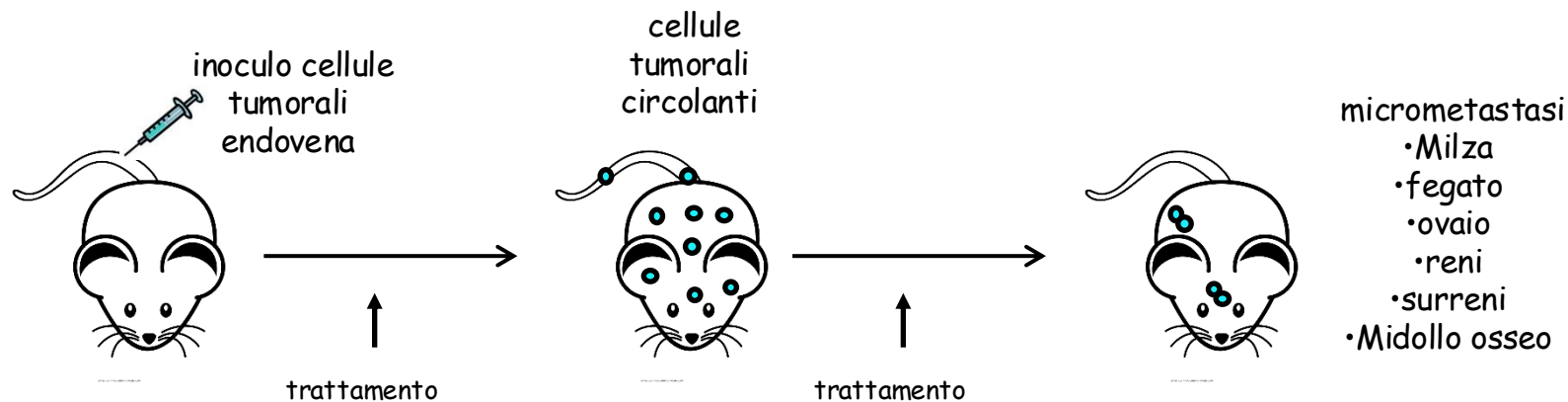
GEMMS
modelli geneticamente modificati transgenici

TH-MYCN: HR-NB, elevata incidenza tumorale/ lungo tempo di crescita, poche metastasi
 TH-MYCN CASP8(KO): metastasi al midollo osseo, elevata incidenza tumorale/ECM alterata
 TH-MYCN p53: perdita inducibile di p53/mutazione di p53 più frequente nelle recidive, sopravvivenza dei topi ridotta
 ALK (F1174): coerente con il fenotipo NB/solo nel 10% dei NB
 TH-MYCN ALK: elevata incidenza tumorale e rapida crescita tumorale/ <10% dei pazienti
 SV40 Tag: fenotipo NB, elevata incidenza tumorale, metastasi/morte media entro 28 settimane

PDX/zPDX
(Patient-Derived Xenografts) in topi NSG/zebrafish



Modello NB pseudo-metastatico

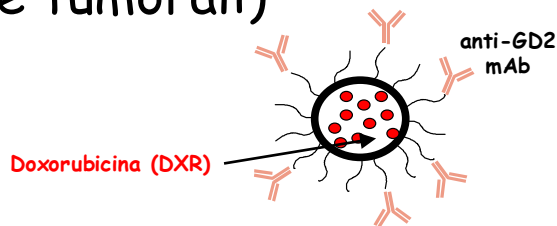
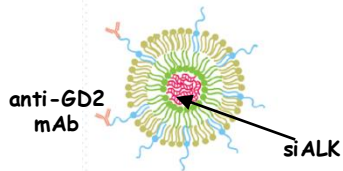


Terapie SofC e sperimentali (es. **targeting attivo tumorale** su cellule circolanti e su micrometastasi indotte)
[i.e., Ab, ADC, NB-targeting NPs]

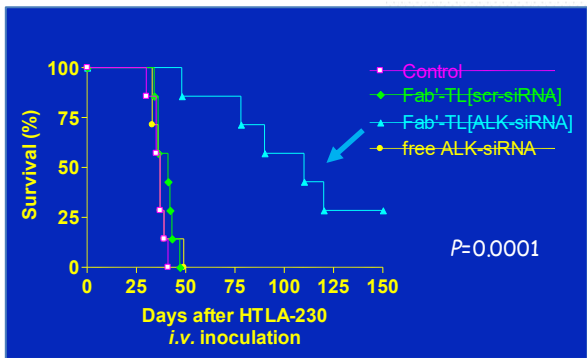
Terapie su modello NB pseudo-metastatico (target: cellule tumorali)

NB esprime di disialoganglioside GD2

Gene ALK: over-espressione, amplificazione o mutazione, sono associate a prognosi infausta e stadi avanzati nel NB

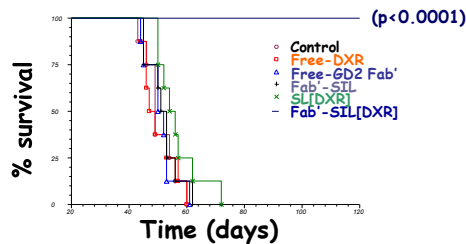


Inibizione metastasi



Aumento sopravvivenza

Survival of Neuroblastoma (HTLA-230)-bearing mice (5 mg DXR / kg mouse; i.v. t 1, 3 days)



Aumento sopravvivenza

	Control	Free-DXR	Free-GD2 Fab'	Fab'-SIL	SL[DXR]	Fab'-SIL[DXR]
Ovaries	50-60 ^a	60-70	40-50	60-60	50-60	0-10
Kidneys	50-50	50-60	40-50	50-50	50-60	0-10
Adrenal glands	30-40	20-30	20-30	30-40	30-40	0-0
Lymphonodes	40-40	20-30	30-40	30-30	20-30	0-0
Bone marrow	30-30	30-40	20-30	30-40	30-30	0-0
Spleen	0-10	10-10	0-10	0-10	0-10	0-0

Pastorino F, Cancer Research 2003a

Di Paolo D, Molecular Therapy 2011

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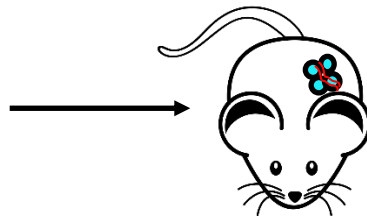
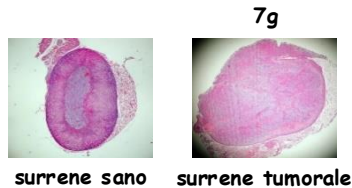
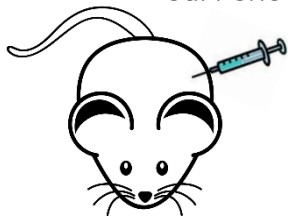
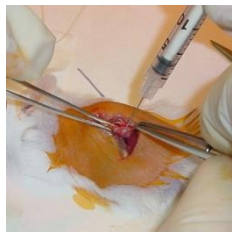


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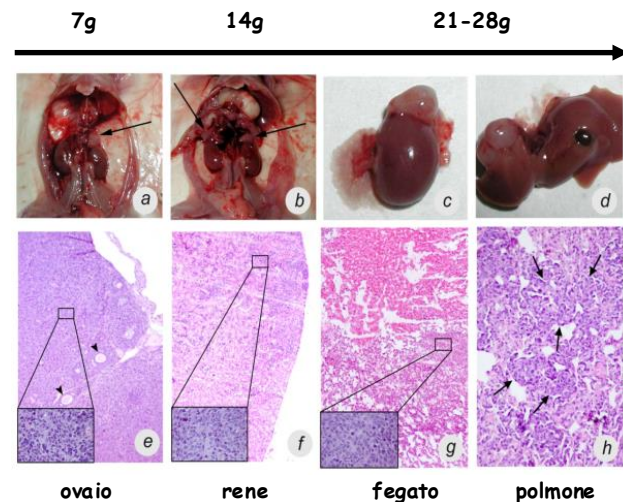


Modello NB ortotopico

inoculo
cellule
tumorali
surrene



treatment massa
tumorale 1°
stabilizzata
+ metastasi

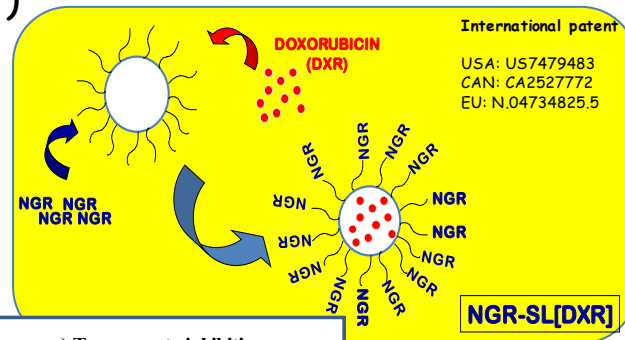


Terapie SofC e sperimentali (es. **targeting attivo tumorale e endotelio tumorale** su massa stabilizzata e metastasi)
[Ab, ADC, NB-targeting NPs]

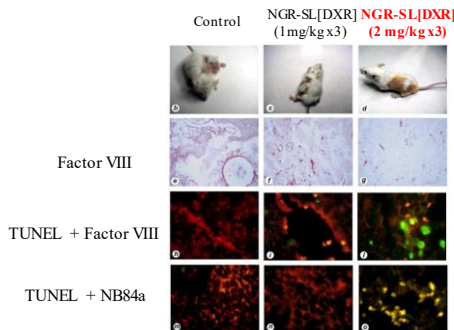
Terapie su modello NB ortotopico (target: cellule endoteliali tumorali)

Differenti isoforme dell'epitopo CD13 (Aminopeptidasi N-APN) sono presenti selettivamente in cellule endoteliali e nei vasi di neo formazione

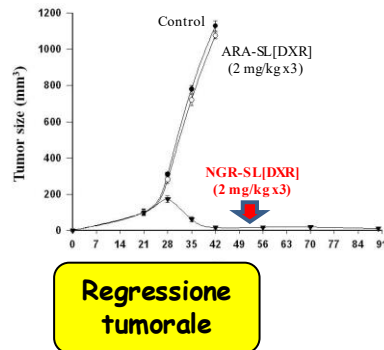
Il peptide **NGR** si lega specificamente all'isoforma del CD13 espressa dai vasi neoangiogenici tumorali



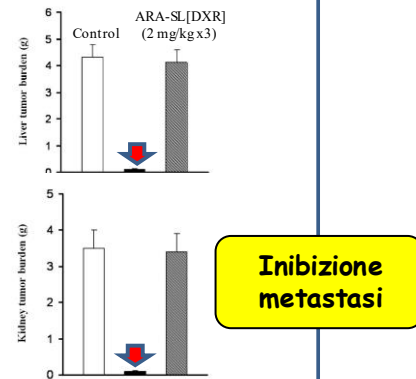
a) Tumor and tumor vasculature inhibition

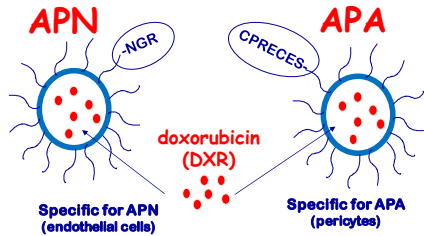


b) Primary tumor growth regression



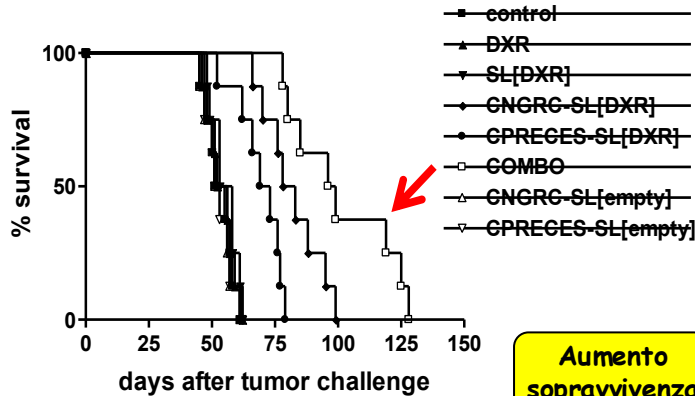
c) Tumor mets inhibition



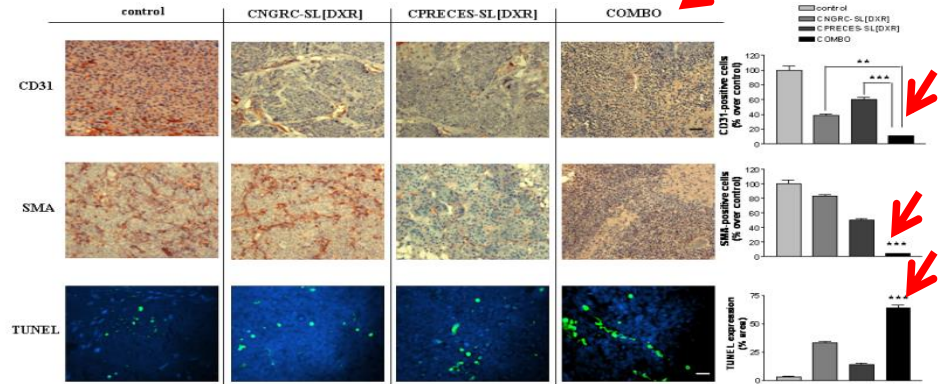


Terapie su modello NB ortotopico (target: cellule endoteliali tumorali e periciti tumorali)

a) Tumor inhibition by endothelial and perivascular tumor targeting (COMBO)



b) Tumor endothelial and pericytes inhibition



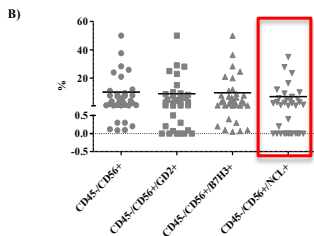
Terapie su modello NB ortotopico (target: cellule tumorali e endoteliali tumorali)

NCL di superficie cellulare

Tumori NB

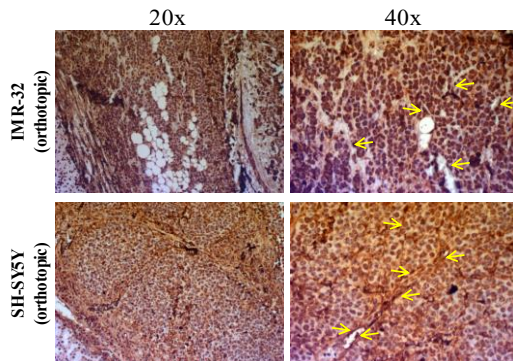
A) Percentage of Cell surface NCL expression in patient-derived NB tumors

Samples	Patient code	Stage	CD45-CD56+ (%)	CD45-CD56+GD2+ (%)	CD45-CD56+87-133+ (%)	CD45-CD56+ NCL+ (%)
Tumor#1	4654	M (relapse)	97	96	96	95
Tumor#2	4668	L2 (relapse)	95,6	94	92	29
Tumor#3	4736	M (relapse)	98,5	92	92	93

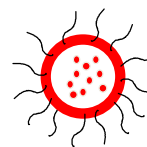


Cellule di NB infiltranti il midollo osseo

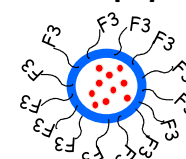
NCL su cellule tumorali e su cellule endoteliali tumorali



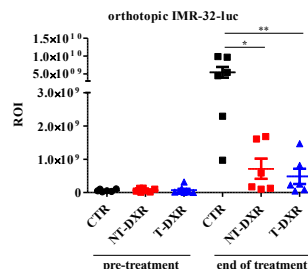
NT: NP[DXR]



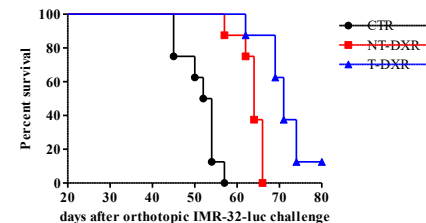
T: F3-NP[DXR]



F3: peptide NCL



**Rallentamento
crescita
tumorale**

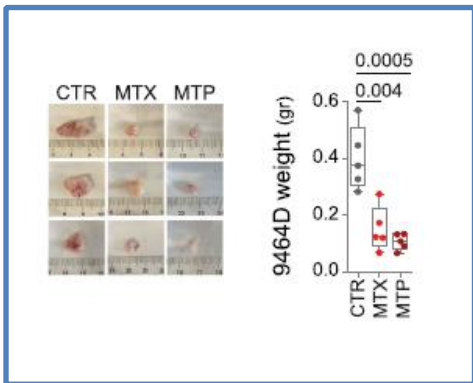


**Aumento
sopravvivenza**

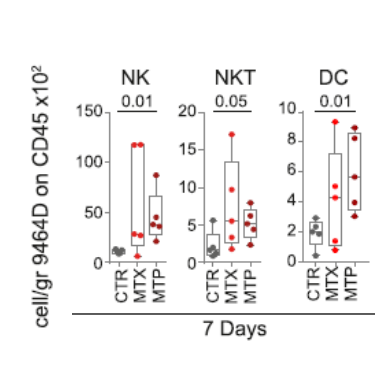
Terapie su modello singenico NB ortotopico

MTX: mitoxantrone

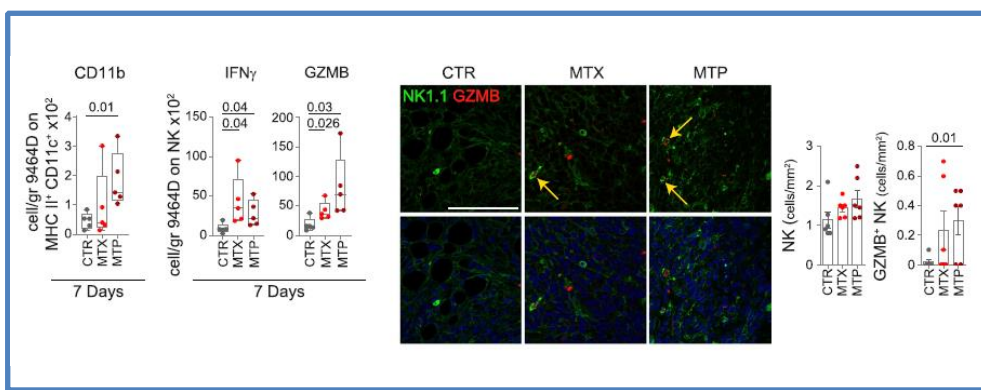
MTP: MTX + anti-TGFb + anti-PD-1



Rallentamento crescita tumorale

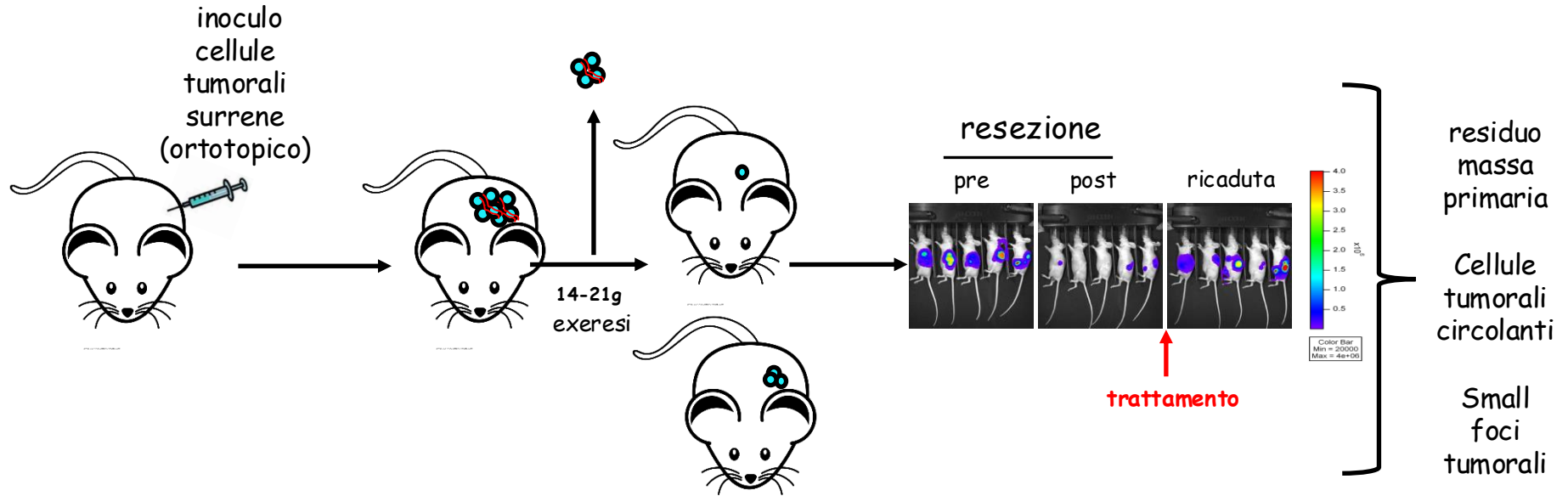


Incremento intratumorale infiltrato linfoide e mieloide



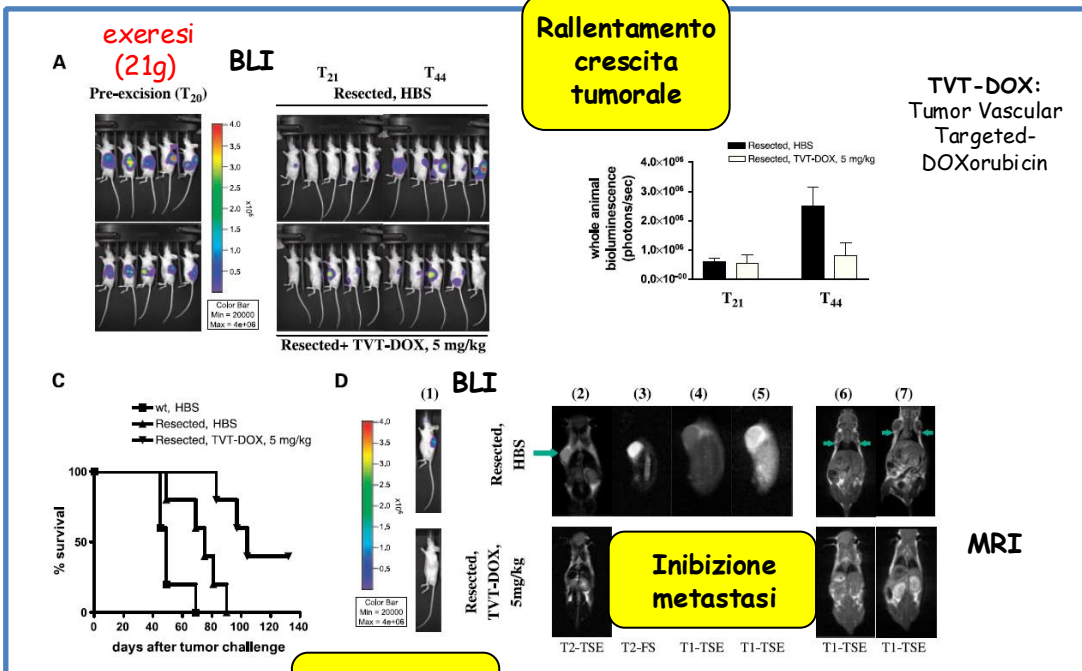
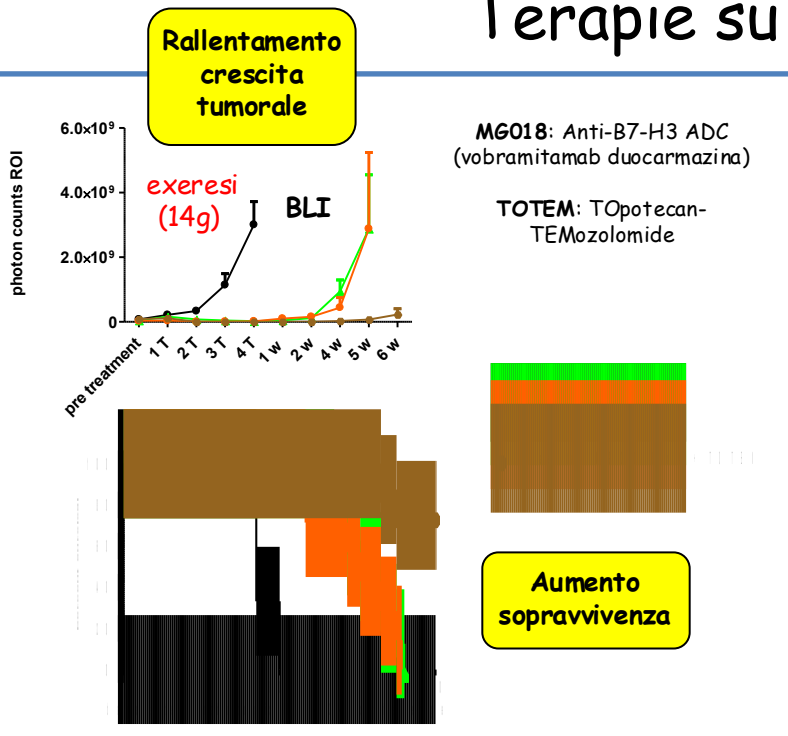
Incremento intratumorale DC e NK attivate

Modello NB «resecato»



Terapie SofC e sperimentali (es. **targeting attivo tumorale e endotelio tumorale** su "malattia residua minima")
[Ab, ADC, NB-targeting NPs]

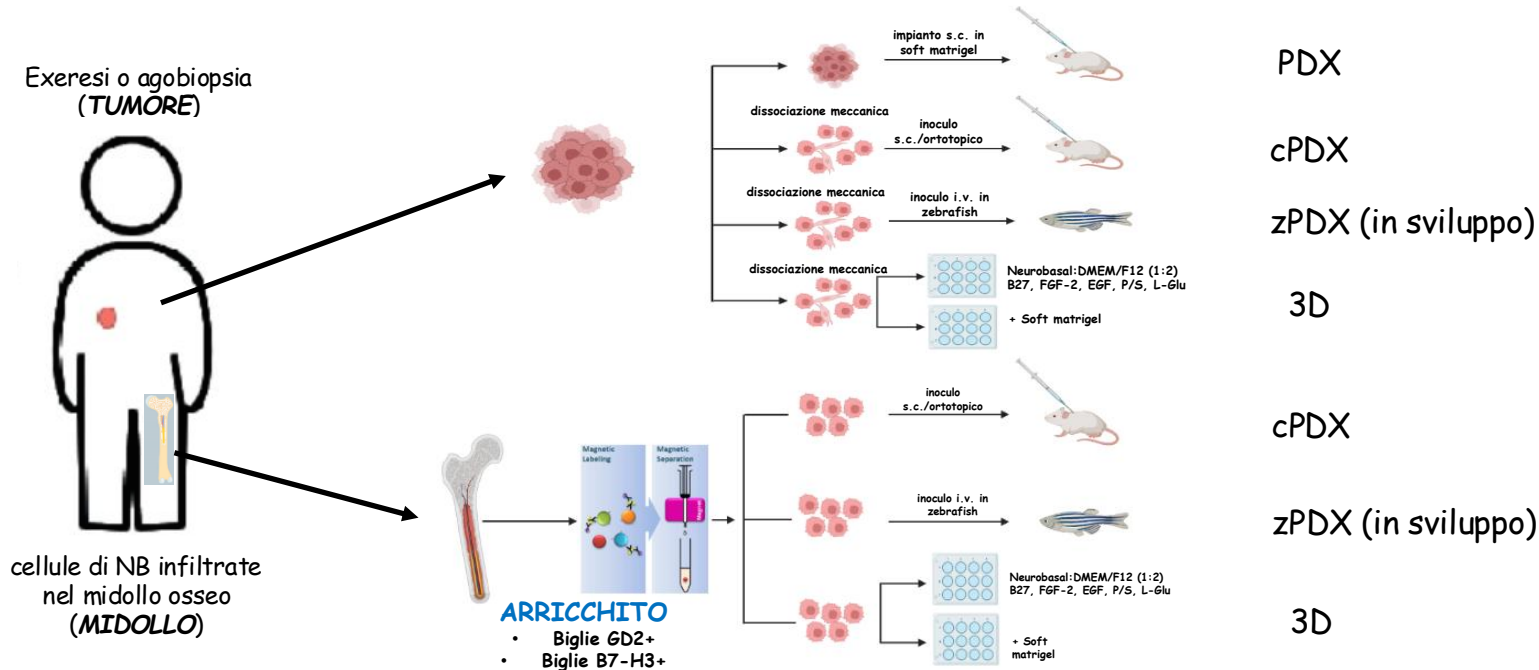
Terapie su modello NB «resecato»



Brignole C, J Immunother Cancer 2023

Pastorino F, Clin Cancer Res 2008

Modello Patient-Derived Xenograft e 3D di NB (PREME)



Modello Patient-Derived Xenograft e 3D di NB

Capasso et al. *Journal of Translational Medicine* (2024) 22:131
<https://doi.org/10.1186/s12916-024-04984-w>

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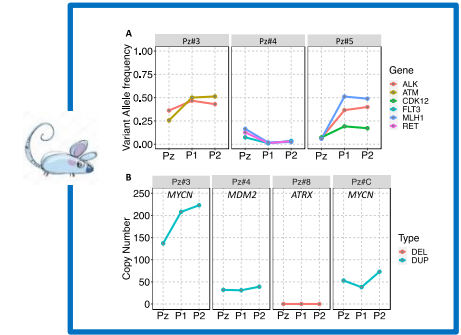
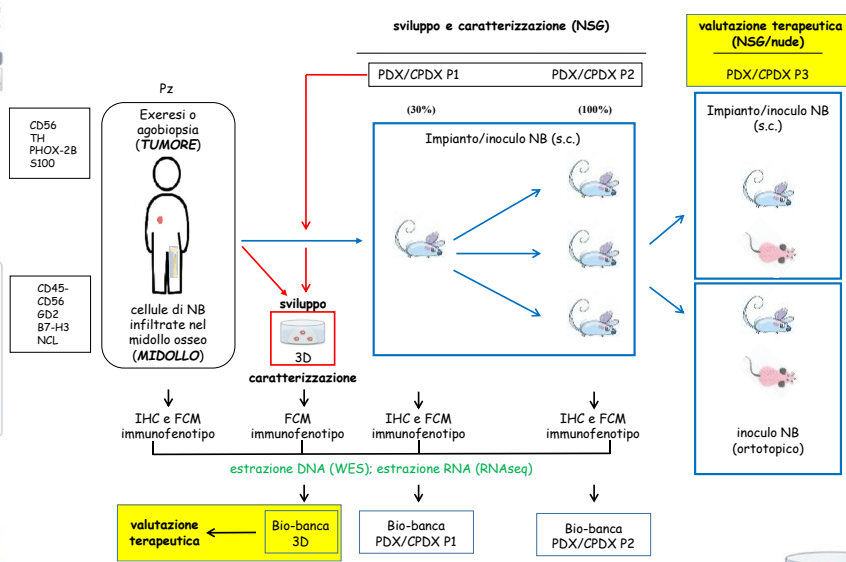
From the identification of actionable molecular targets to the generation of faithful neuroblastoma patient-derived preclinical models

Mario Capasso^{1*}, Chiara Brignone¹, Vito A. Lazara¹, Veronica Benzi¹, Susa Cantalupo^{1,3}, Enrico Sebastiani¹, Alessandro Quattrone¹, Eleonora Ciampi¹, Marianna Avitabile^{1,4}, Angela R. Sementa¹, Katia Mazzocco¹, Barbara Carletta¹, Gabriele Gaggero¹, Valerio G. Veltono¹, Michele Citi¹, Enzo Calarco¹, Ilma Gusto¹, Patrizia Perri¹, Sang Aweel¹, Daniela Fusi¹, Analia Tassi¹, Roberto Luchini¹, Rosella Murzi¹, Marco Rabusin¹, Francesco De Leonardis¹, Monica Cellini^{1,5}, Paola Coccia¹, Achille Iakouss¹, Maria V. Comas¹, Massimo Comi¹, Alberto Garaventa¹, Loreana Amoroso¹, Mirco Pozzoni^{1,6} and Fabio Testoni^{1*}

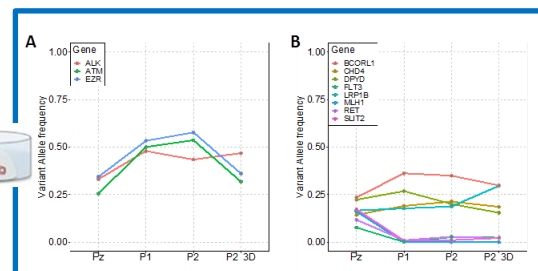
Abstract
Background: Neuroblastoma (NB) represents the most frequent and aggressive form of embryonal solid tumor of infants. Although the overall survival of patients with NB has improved in the last years, more than 50% of high-risk patients still undergo a relapse. Thus, in the era of precision/personalized medicine, the need for high-risk NB patient-specific therapies is urgent.
Methods: Within the Personalized Medicine (PRIME) program, patient-derived NB tumors and bone marrow (BM)-infiltrating NB cells, derived from either live cells or tumor bone biopsies, underwent to histological and to flow cytometry immunophenotyping, respectively. BM samples containing a NB cells infiltration from 1 to 50 percent, underwent to a subsequent NB cells enrichment using immune-magnetic manipulation. Then, NB samples were used for the identification of actionable targets and for the generation of 3D tumor-spheres and Patient-Derived Xenografts (PDX) and Cell PDX (CPDX) preclinical models.
Results: Eighty-four percent of NB patients showed potentially therapeutically targetable alterations (including copy number mutations, copy number variations and mRNA over-expression). Sixty-six percent of samples showed alterations, graded as "very high priority", that are validated to be directly targetable by an approved drug or an investigational agent. A molecular targeted therapy was applied for four patients, while a genetic counseling was suggested to two patients having one pathogenic germline variant in known cancer predisposition genes. Out of eleven samples implanted in mice, five gave rise to CPDX. All preserved in a local PDX Bio-bank. Interestingly, comparing all

* Mario Capasso, Chiara Brignone and Vito A. Lazara have the same first authorship.
 Mario Pozzoni and Vito A. Lazara share the last authorship.
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 mario.pozzoni@univr.it
 full list of author information is available at the end of the article

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Varianti somatiche patogene e CNV conservate



Gastlini

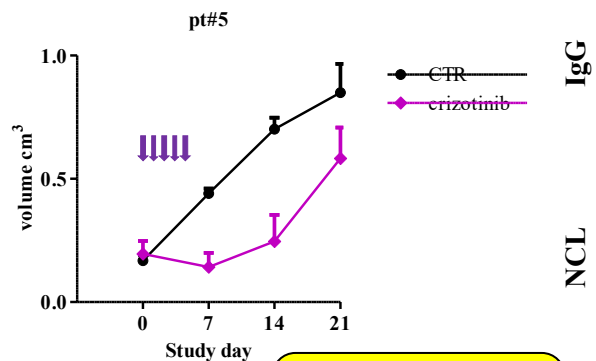


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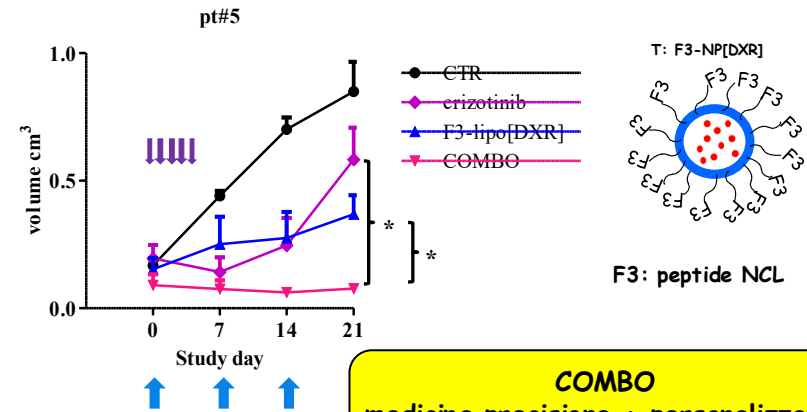
Capasso M, *J Transl Medicine* 2024



PDX da biopsia pt NB con mutazione ALK (PREME)



Medicina molecolare (precisione) rallenta crescita tumorale



COMBO medicina precisione + personalizzata: Stop crescita

Modelli NB in sviluppo

Zebrafish

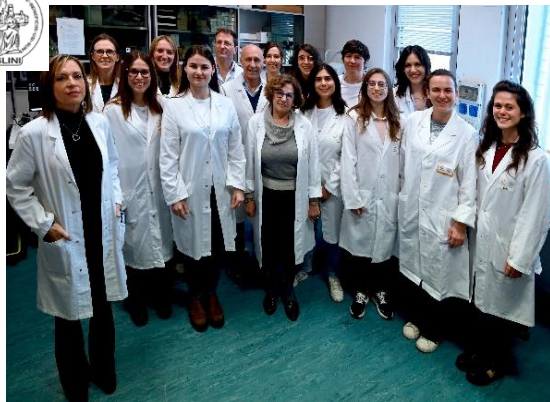
Impianto/inoculo di frammenti tumorali o cellule (CM-Dil) da paziente nello spazio perivitellino. Screening e visualizzazione rapida risposta alle terapie anti-tumorali, anti-metastatiche e anti-angiogeniche (i.e. vascolatura egfp)

Modello metastasi ossee

Inoculo cellule NB direttamente nell'osso (sito di elezione metastasi NB)

Umanizzato on-running

Impianto frammenti tumorali e cellule da pazienti NB in topi con sistema immunitario umano
Valutazione combinazioni terapeutiche immuno-oncologiche



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Pazienti e famiglie

Centri Oncologici AIEOP

Massimo Conte
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Filippo Spreafico
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FONDAZIONE AIRC
PER LA RICERCA SUL CANCRO ETS



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