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Dana-Farber
Cancer Institute

UNIVERSITÀ
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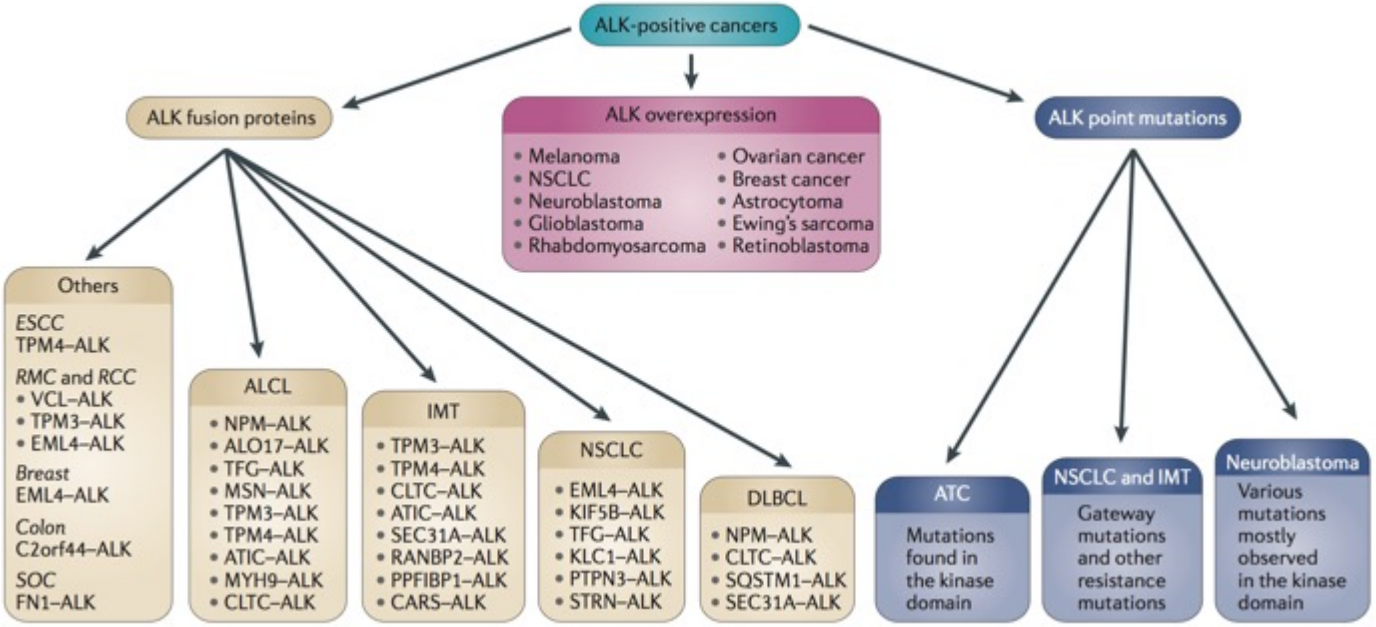
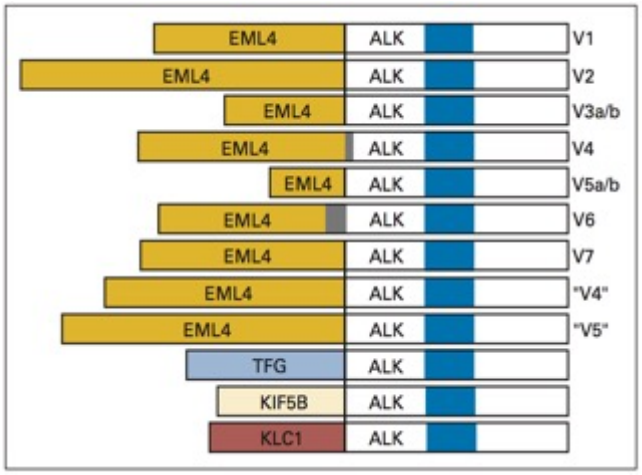
IEO
Istituto Europeo
di Oncologia

An immune portfolio for ALK tumors: from vaccine to cellular immunotherapies

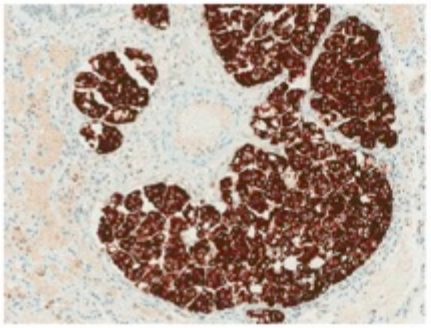
Roberto Chiarle, MD

ALK IN CANCERS

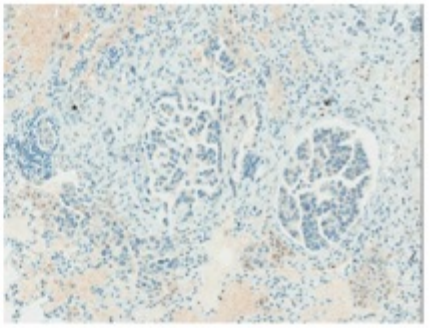




ALK-REARRANGED NSCLC



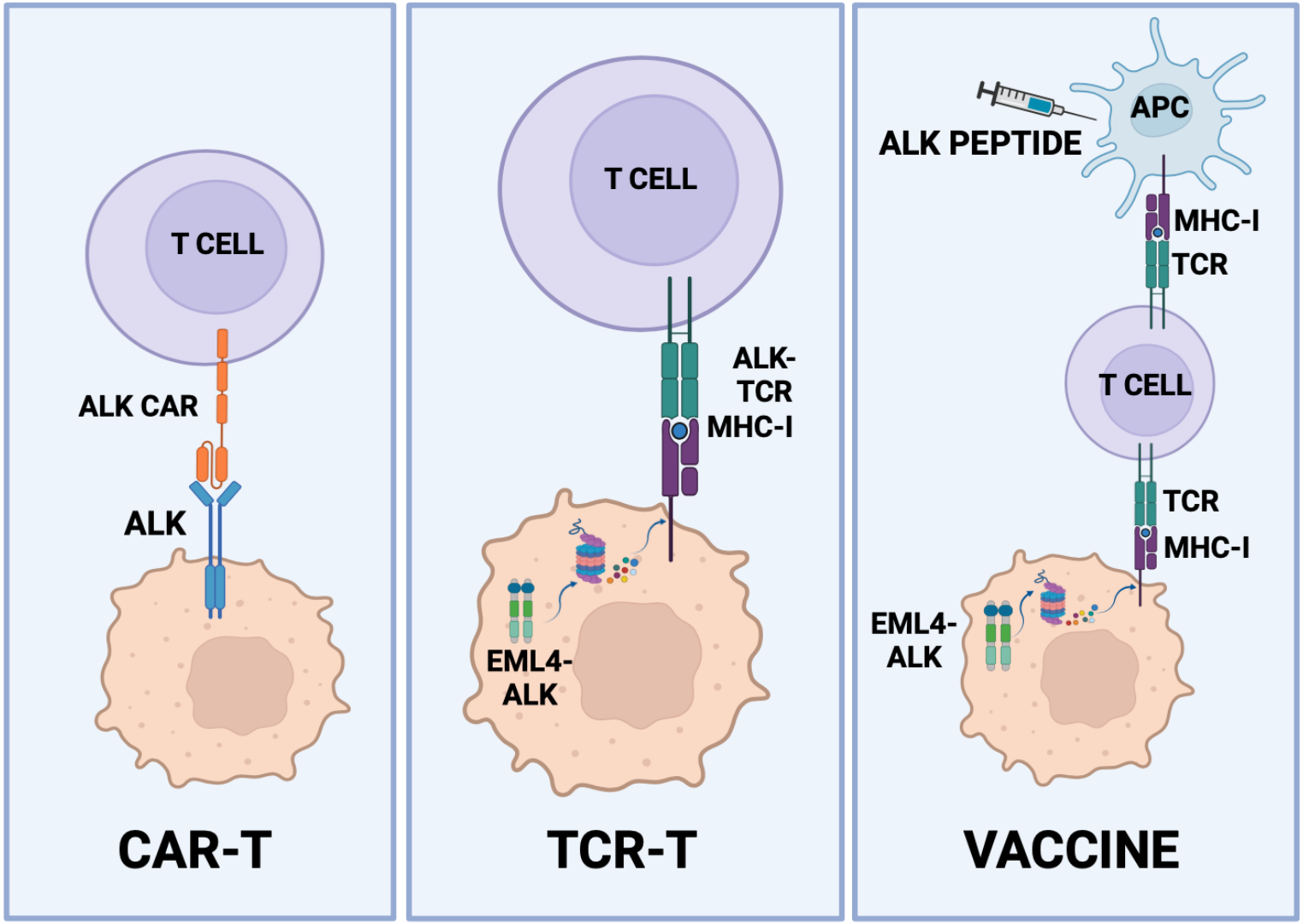
ALK-NEGATIVE NSCLC



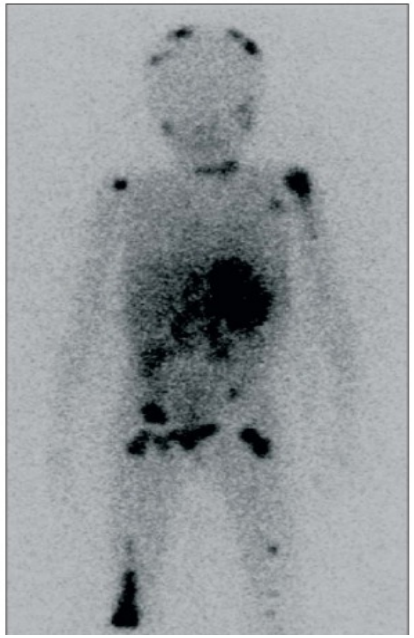
Key immunological properties of ALK as an oncoantigen

- Restricted and low levels of expression in normal tissues
- Selective and high expression by the tumor cells
- Strong addiction of the neoplastic cells on the ALK signalling for their growth and survival
- Good spontaneous immunogenicity of the ALK protein in human patients

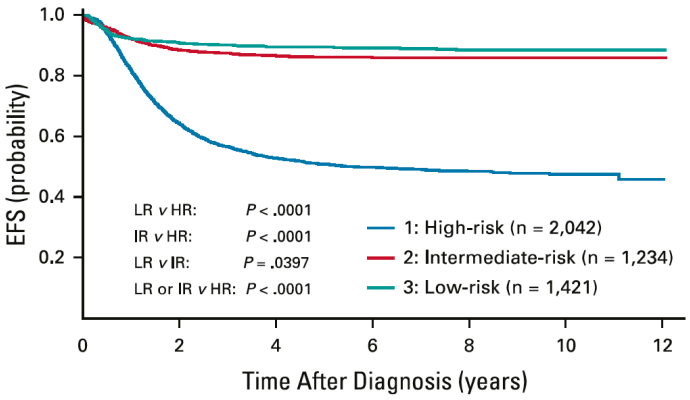
Portfolio of anti-ALK immunotherapies



Pediatric neuroblastoma (NBL)

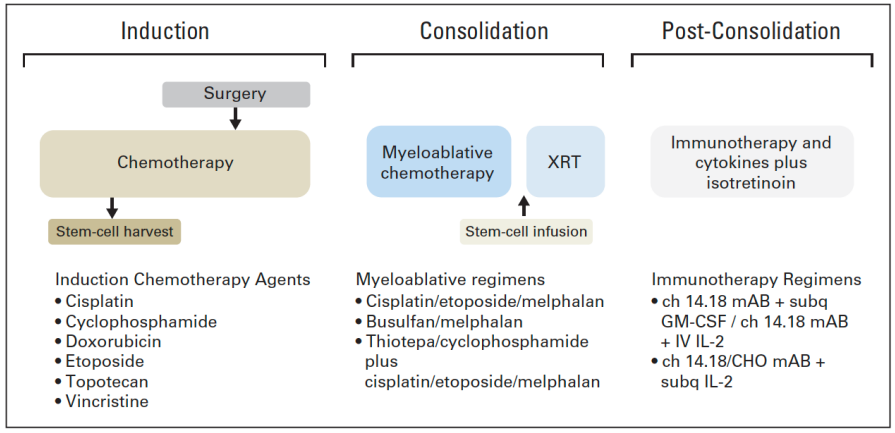


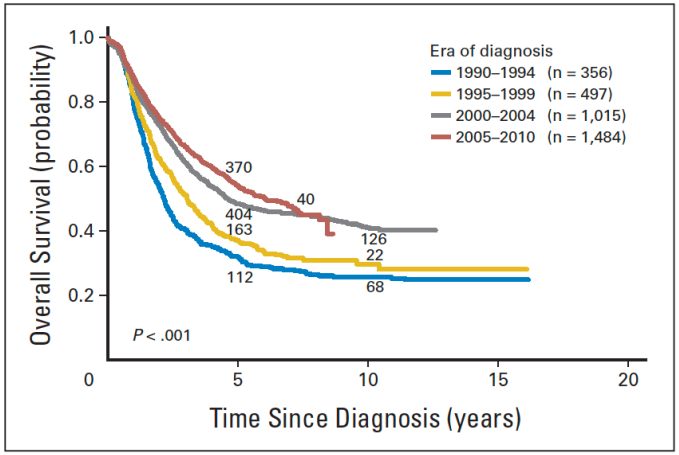
- Most common extra-cranial solid tumor in childhood
- Accounts for ~ 15% of pediatric cancer deaths
- Outcomes for LR and IR neuroblastoma are favorable
- 50% of patients present with high-risk features
- Outcomes for HR neuroblastoma remain poor despite intensive multimodal therapy



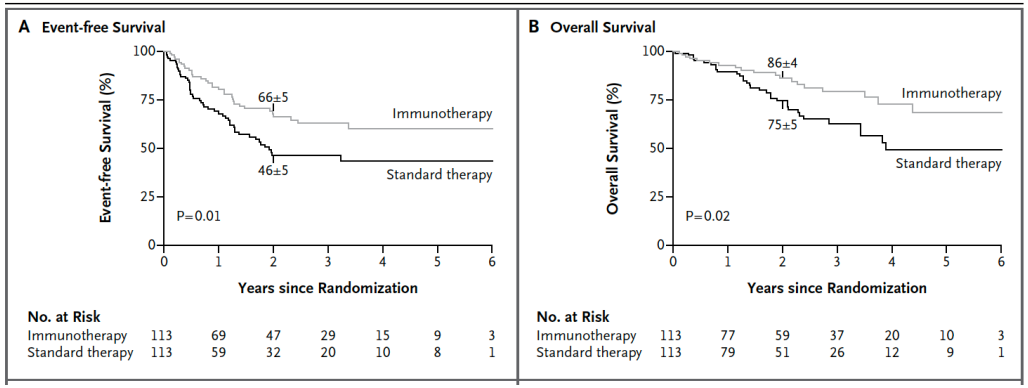
Irwin et al, JCO 2021

Pediatric HR neuroblastoma



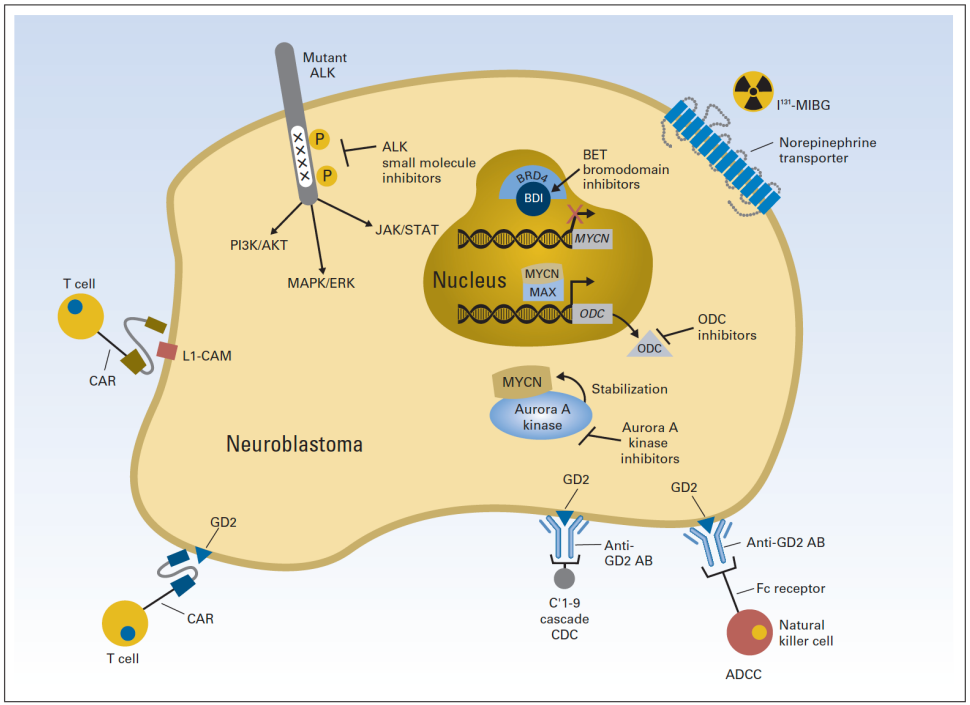


Pinto N et al, JCO 2015



Yu A et al, NEJM 2010

Therapeutic approaches for HR neuroblastoma

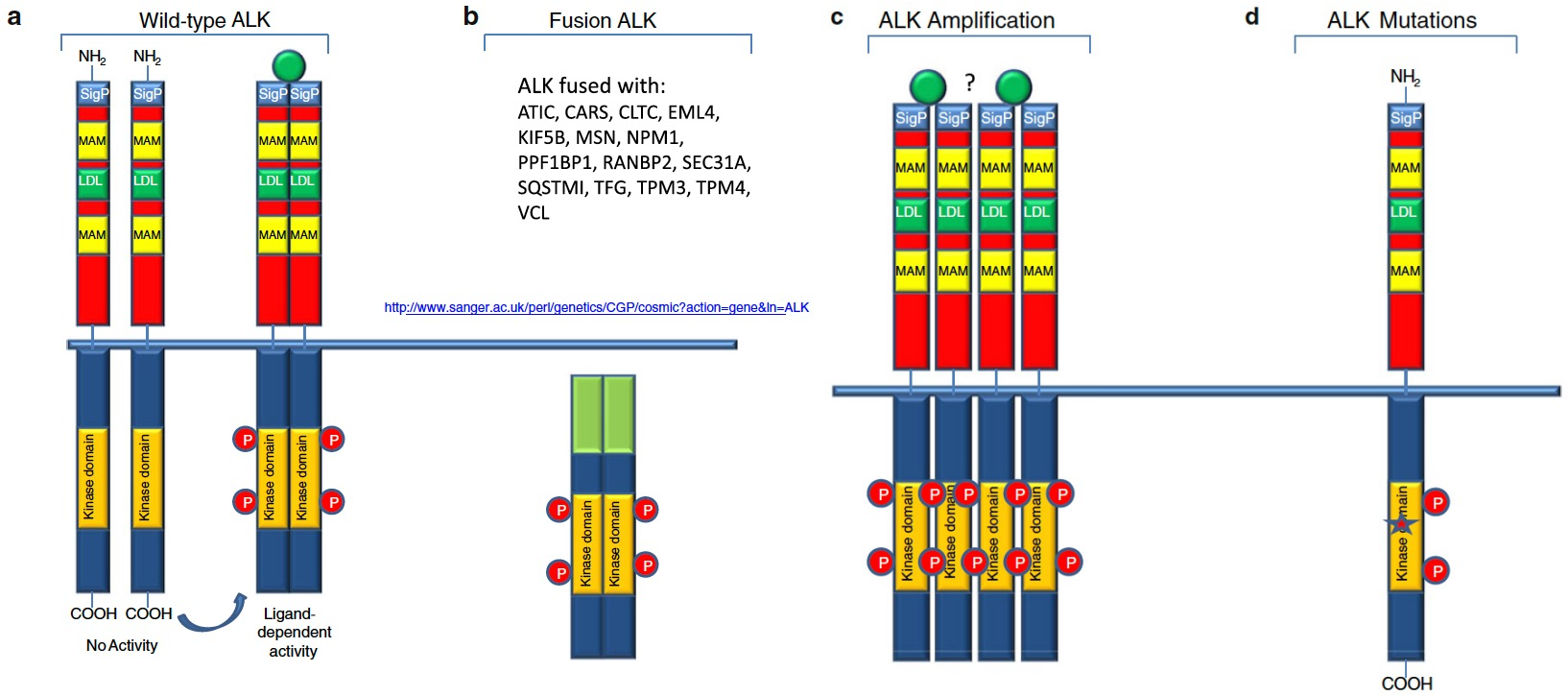


Pinto N et al, JCO 2015

- ~ 50% of HR NBL patients relapse or fail to respond to upfront therapy
- Outcomes for r/r HR neuroblastoma are dismal (4y PFS 6%, 4y OS 20%)
- Novel therapeutic approaches for r/r neuroblastoma are urgently needed

London W. et al, Cancer 2017
DuBois S et al, ASCO Education 2022

Targeting ALK in neuroblastoma



~90% NBL

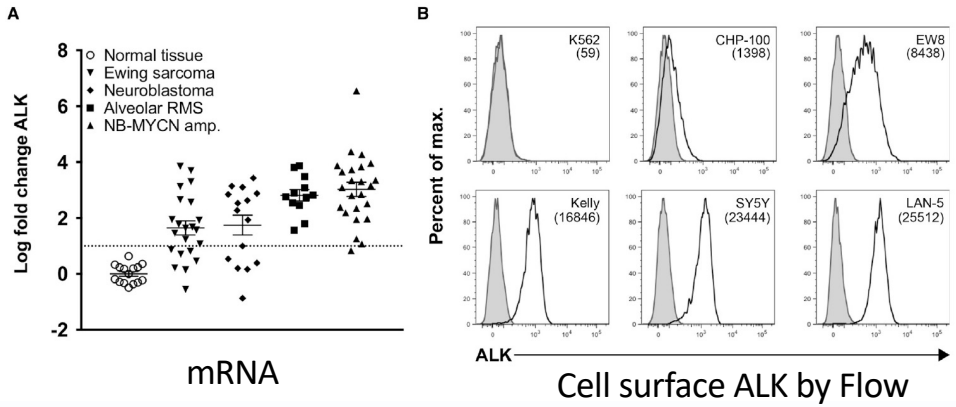
NSCLC, ALCL

~5% NBL

~10% NBL

Morales La Madrid A et al., Targ Onc 2012

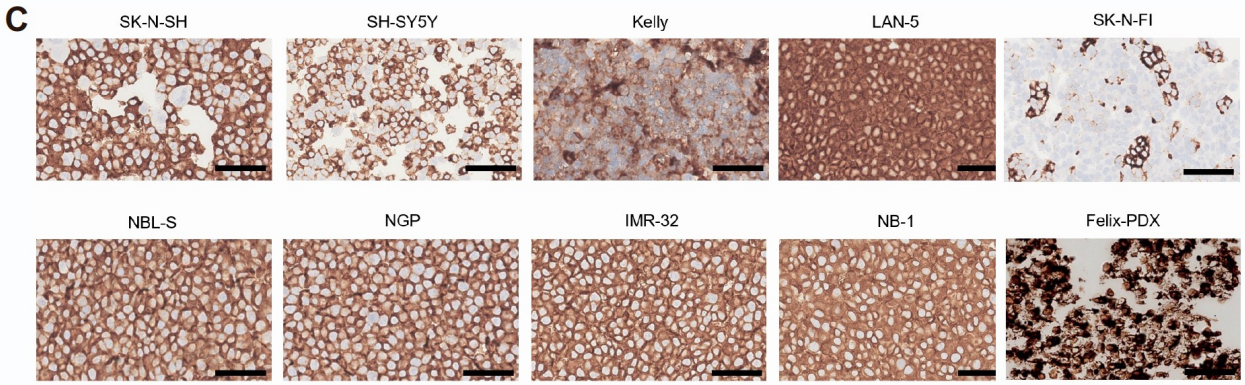
ALK surface-expression in neuroblastoma



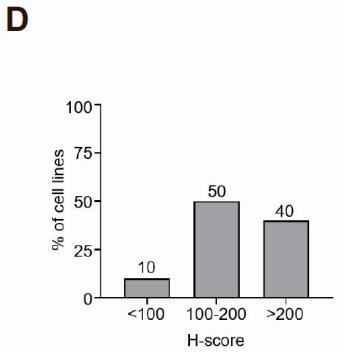
mRNA

Cell surface ALK by Flow

Walker AJ et al, Mol Ther 2017



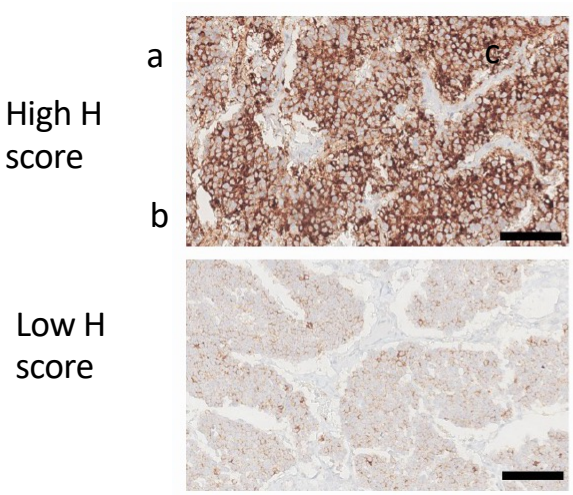
Representative IHC staining with ALK mAb on NBL cell lines

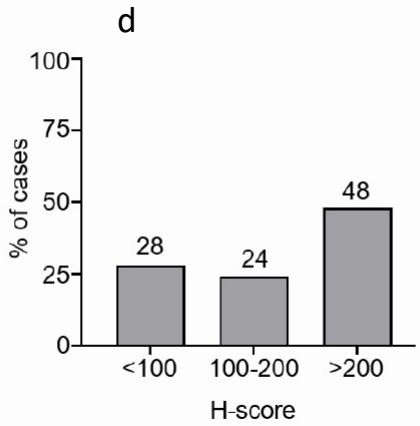


IHC Score summary

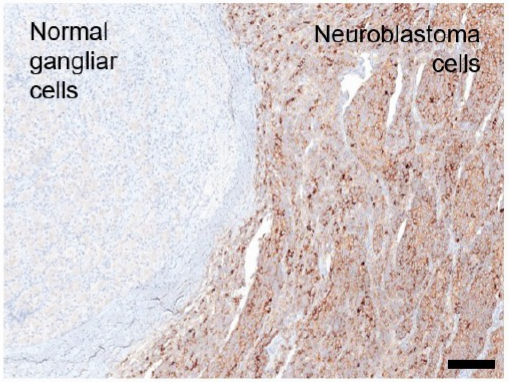
Bergaggio, E et al, Cancer Cell 2023

ALK expression by IHC in primary pediatric NBL





Summary of IHC on 50 primary pediatric NBL samples

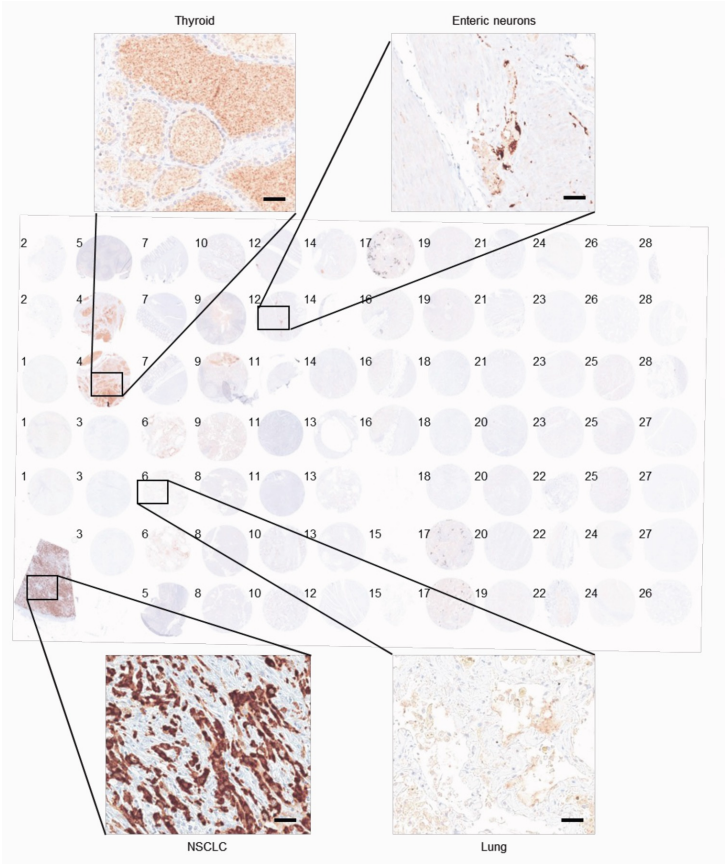


Example of ALK expression in normal gangliar cells vs. NBL

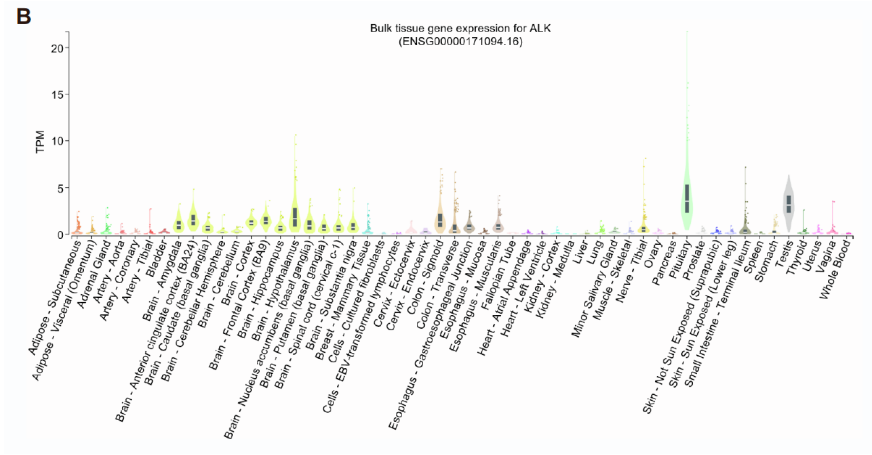
Bergaggio, E et al, Cancer Cell 2023

ALK expression in healthy tissues

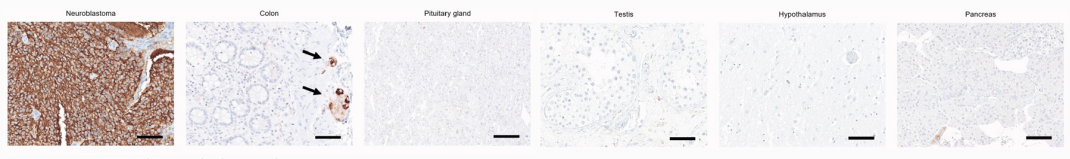
TMA (D5F3 FDA approved mAb)



Number	Tissue	Number	Tissue	Number	Tissue	Number	Tissue
1	liver	8	pancreas	15	breast	22	gallbladder
2	skin	9	appendix	16	stomach	23	myocardium
3	cerebral cortex	10	placenta	17	lymph node	24	adrenal gland
4	thyroid gland	11	bladder	18	ovary	25	hypophysis
5	tonsillar tissue	12	colon	19	spleen	26	testicle
6	lung	13	prostate	20	uterus	27	hypothalamus
7	duodenum	14	kidney	21	uterine tube	28	ileum



mRNA
by GTEx



IHC on healthy donor tissues

Bergaggio, E et al, Cancer Cell 2023

Targeting ALK in neuroblastoma

- ~10% ALK mutated;
- ~3-5% ALK amplified
- ~90% ALK expressed on the cell surface (>72% at moderate or high levels)

- High ALK expression associated with adverse clinical outcome

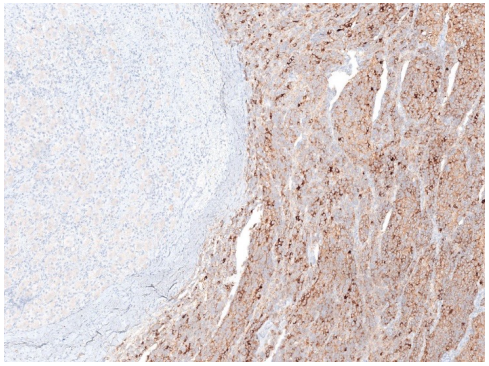
- Expression of ALK is restricted to tumor cells

- ALK tyrosine kinase inhibitors (lorlatinib) have efficacy in ALK-mutated neuroblastoma and the RP2D for pediatric NBL patients has recently been defined (Goldsmith K, et al., Nature Medicine 2023)

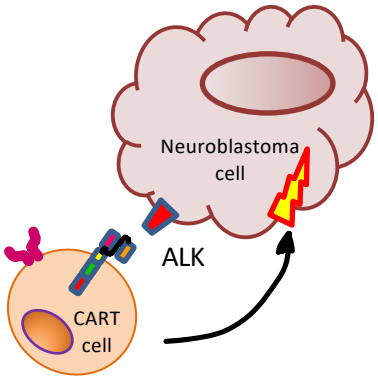
ALK is a promising target for CAR-based T cell therapy

ALK staining (D5F3 FDA approved)

Normal gangliar cells

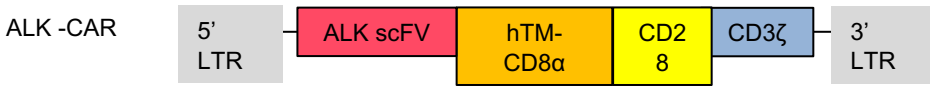


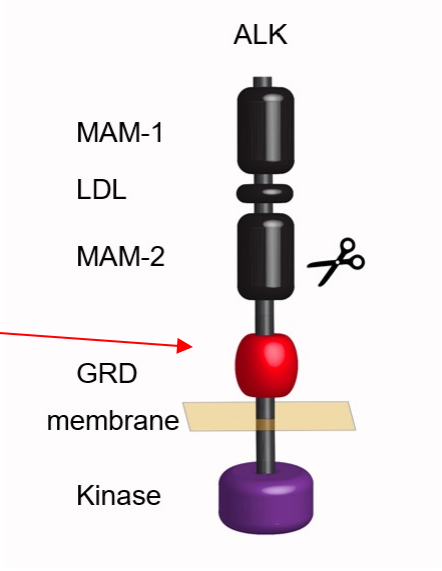
NB cells



ALK-CART cell construct

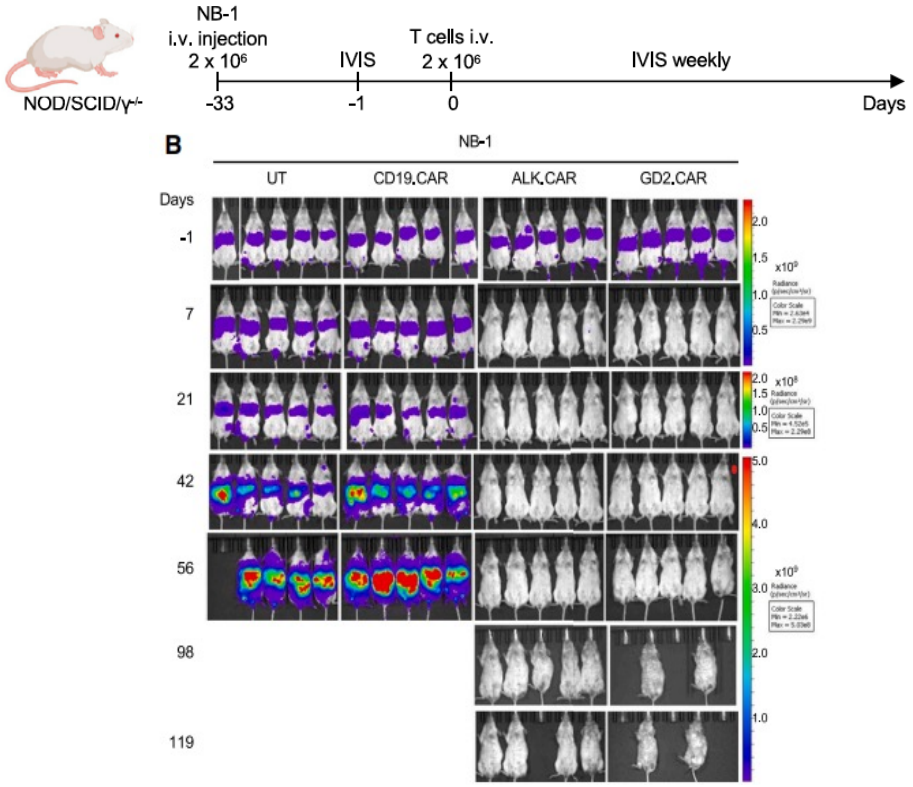
ALK Ab	ALK phosphorylation	$K_{D, app}$ [nM]	ALK turnover	Mouse-ALK binding	KTN#
1	no activity	0.35	no	moderate	118
2	inhibitor	0.2	no	moderate	119
3	weak inhibitor	0.5	no	no	120
4	strong agonist	0.5	strong	no	123
5	inhibitor	0.5	no	strong	125
6	inhibitor	0.4	strong	strong	130
7	weak agonist	0.5	strong	strong	131





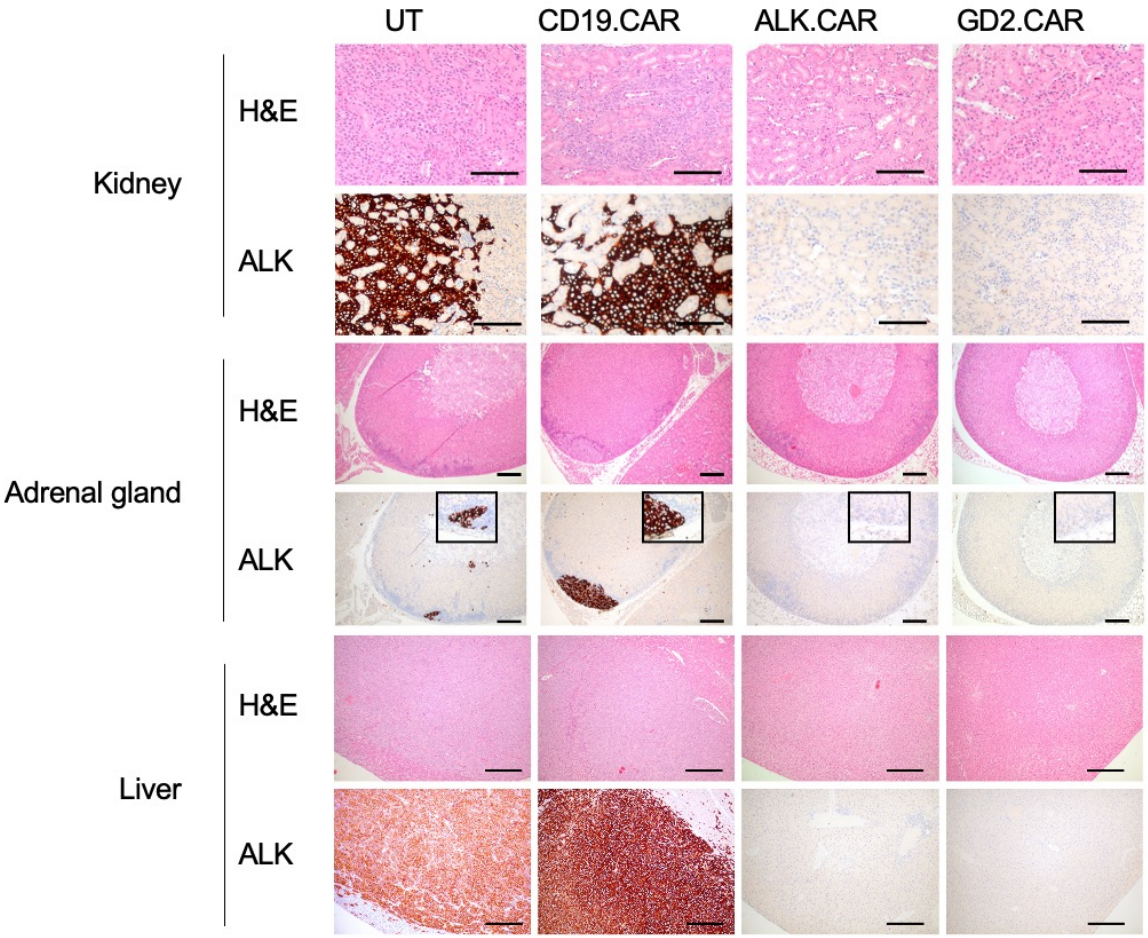
Bergaggio, E et al, Cancer Cell 2023
Huang H, et al, Cell Reports 2021

ALK-CART cells efficiently kill neuroblastoma with high ALK expression (ALK-amplified NB-1 model)



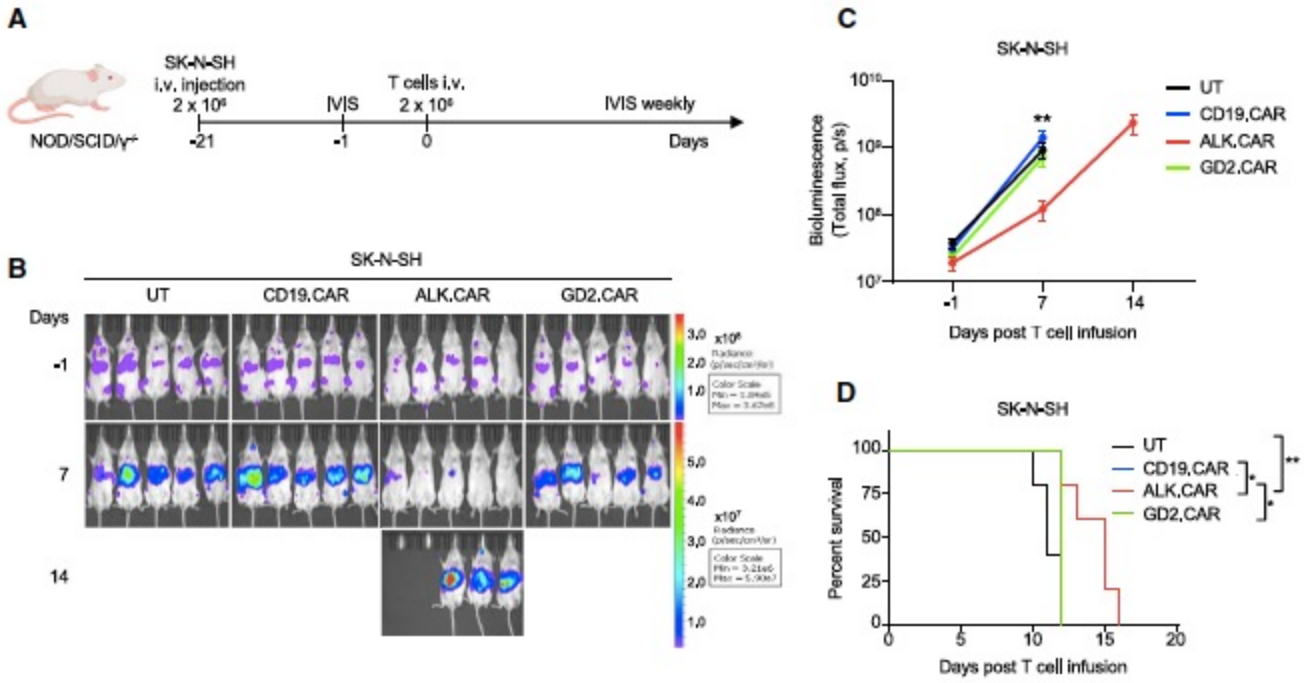
Bergaggio, E et al, Cancer Cell 2023

ALK-CART cells efficiently kill neuroblastoma with high ALK expression (ALK-amplified NB-1 model)



Bergaggio, E et al, Cancer Cell 2023

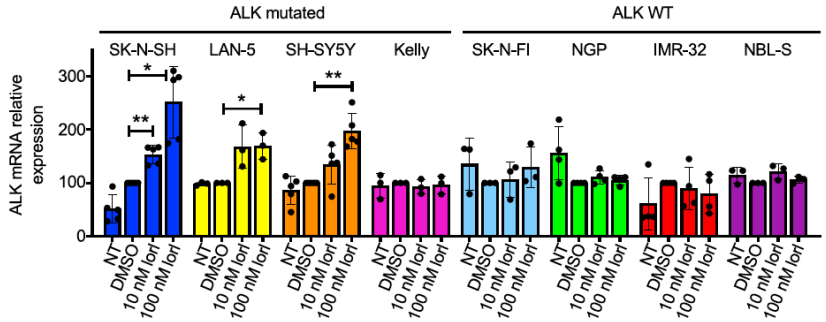
ALK-CART activity is modest in metastatic NBL models with low ALK expression (ALK-mut SK-N-SH)



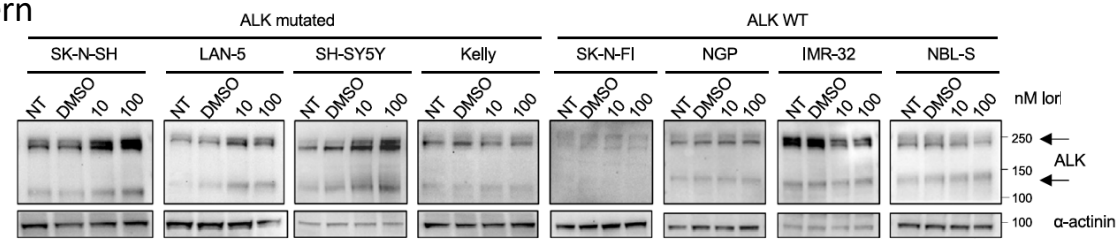
Bergaggio, E et al, Cancer Cell 2023

Lorlatinib enhances ALK expression on the NBL surface

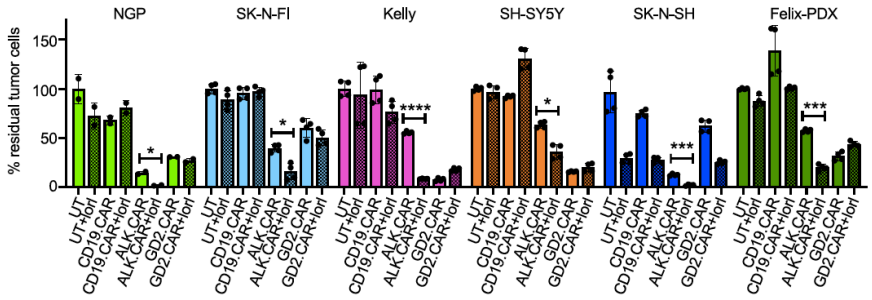
mRNA



Western Blot



Co-culture assay 1:5 ET ratio x 3d



The constitutive activity of the ALK-mutated receptor impairs receptor trafficking

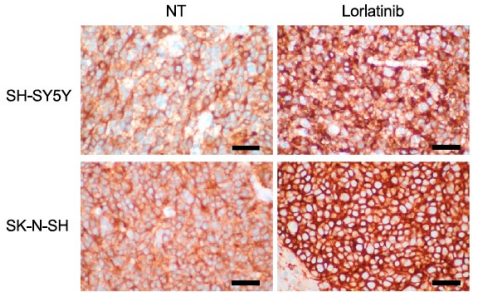
ALK TKI

blockade of ALK signaling

decreased receptor internalization

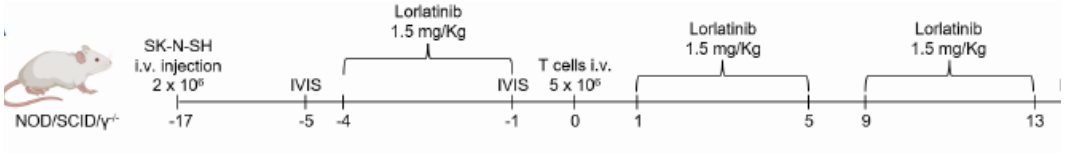
increase ALK transcription

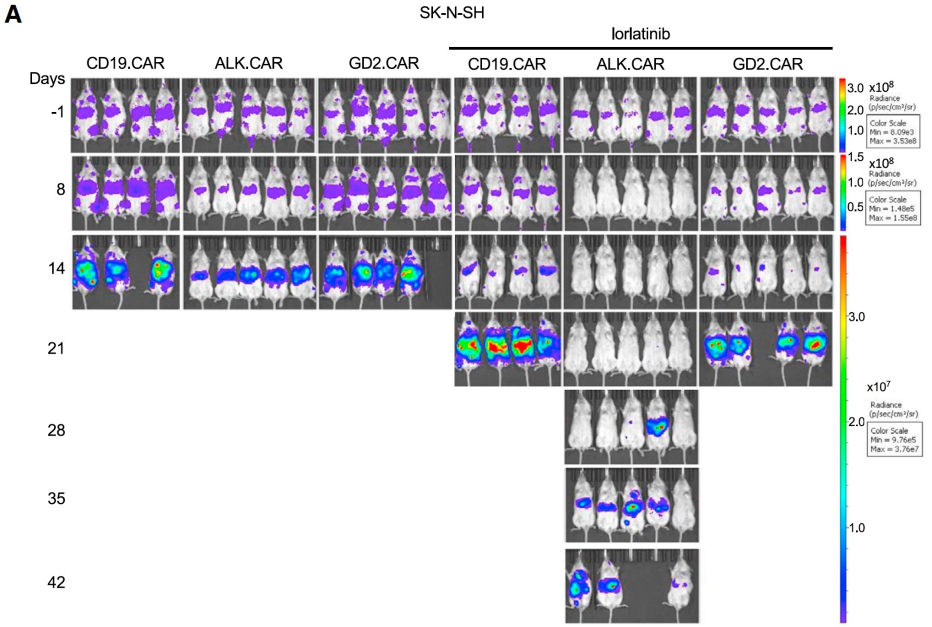
increased cell surface localization

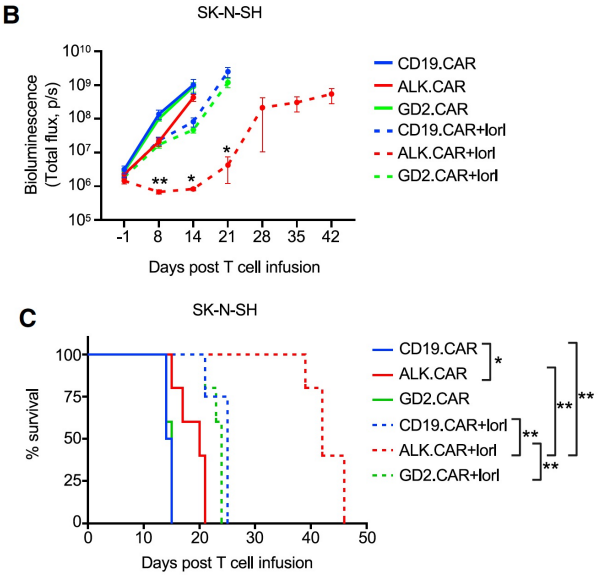


Bergaggio, E et al, Cancer Cell 2023

Lorlatinib enhances ALK-CART efficacy against NBL with low ALK expression (ALK-mut SK-N-SH)



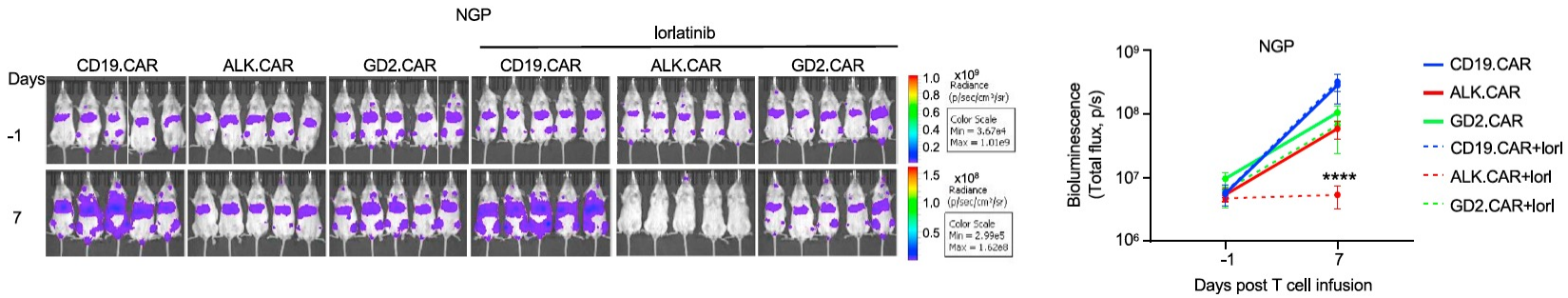




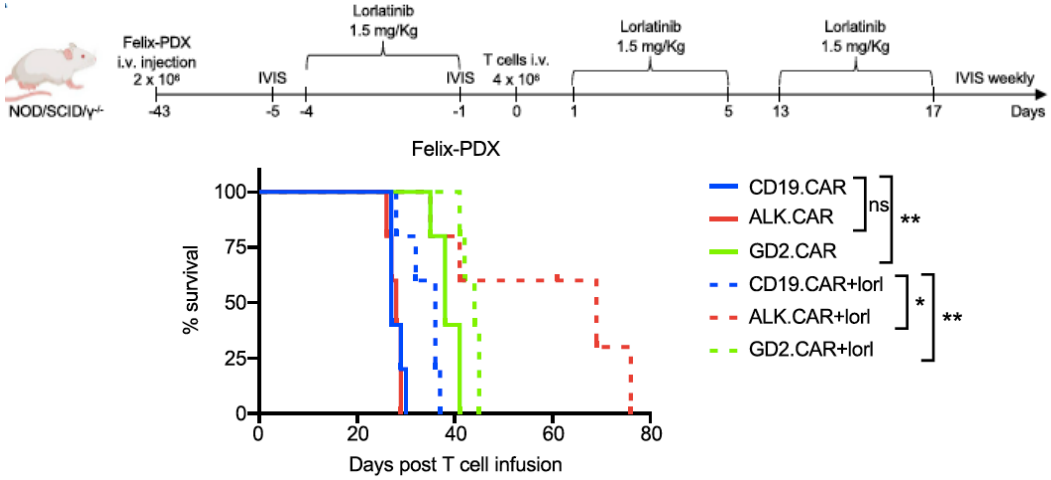
Bergaggio, E et al, Cancer Cell 2023

Lorlatinib enhances ALK-CART efficacy metastatic ALK-WT (NGP) and ALK-mut PDX (Felix models)

NGP



Felix-PDX

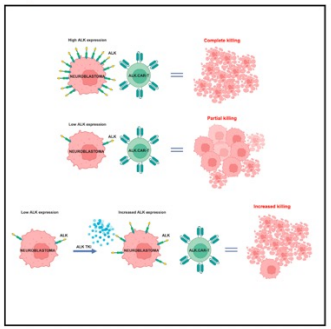


Bergaggio, E et al, Cancer Cell 2023

Cancer Cell

ALK inhibitors increase ALK expression and sensitize neuroblastoma cells to ALK.CAR-T cells

Graphical abstract



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In brief

Bergaggio et al. show that CAR-T cells targeting ALK (ALK.CAR-Ts) eradicate neuroblastoma with high ALK expression, without associated toxicity. In neuroblastoma with low ALK expression, ALK inhibitors increase ALK surface expression while impairing tumor growth, thereby enhancing ALK.CAR-T cell function. Combination of ALK.CAR-T cells and ALK TKI represent a potent double hit approach to target a driver oncogene in neuroblastoma.

ALKemy to enhance chimeric antigen receptor T cell immunotherapy for neuroblastoma

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[2]Department of Medicine, Division of Hematology and Oncology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

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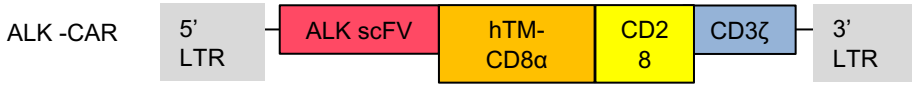
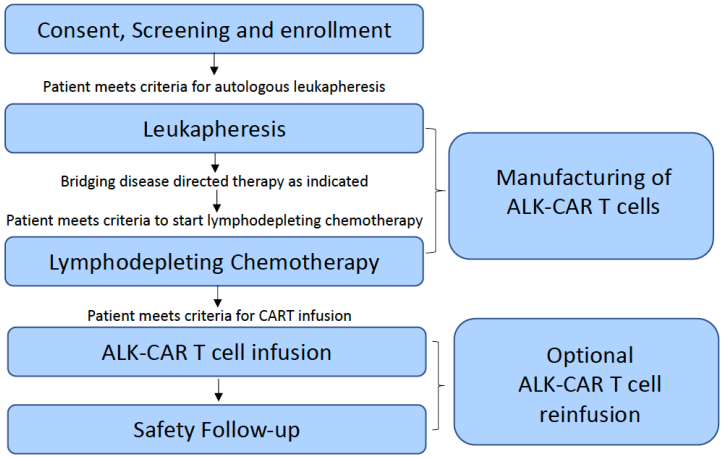
Chimeric antigen receptor (CAR) T cell immunotherapy in solid cancer is severely limited by the absence of ideal targets. In this issue of *Cancer Cell*, Bergaggio et al. find that anaplastic lymphoma kinase (ALK) inhibitors can enhance the function of ALK-specific CAR T cells against neuroblastoma by increasing target density in cancer cells.

The results of chimeric antigen receptor (CAR) T immunotherapy in solid cancers have been dismal so far as compared to that in hematological malignancies, despite the fact that this innovative immunotherapy was first tested in solid cancers. Only a handful of patients out of hundreds treated in clinical trials have reached complete responses, and in most cases these responses were short lived. While several failure mechanisms have been described for CAR T in solid cancer, such as T cell exhaustion, the tumor microenvironment, and intrinsic tumor resistance, a crucial issue is the lack of optimal targets. Moreover, in a subset of patients, severe toxicities have been reported that are in part

ALK-CART Phase I/II Clinical Trial Design



γ-retroviral cGMP vector
(manufactured at Baylor CAGT)



Dose Level	Dose (viable, CAR+ T cells/kg)
-1	$3x10^5$
1	$1x10^6$
2	$3x10^6$
3	$1x10^7$
4	Lorlatinib + $1x10^7$ (or MTD)
Phase 2 Dose expansion at MTD +/- Lorlatinib	
Observe a 28 day DLT window post-infusion prior to dose-escalation	

IND holder: Roberto Chiarle, MD
PI: Susanne Baumeister, MD
Co-PI: Suzanne Shusterman, MD

Anticipated activation in October/November 2024

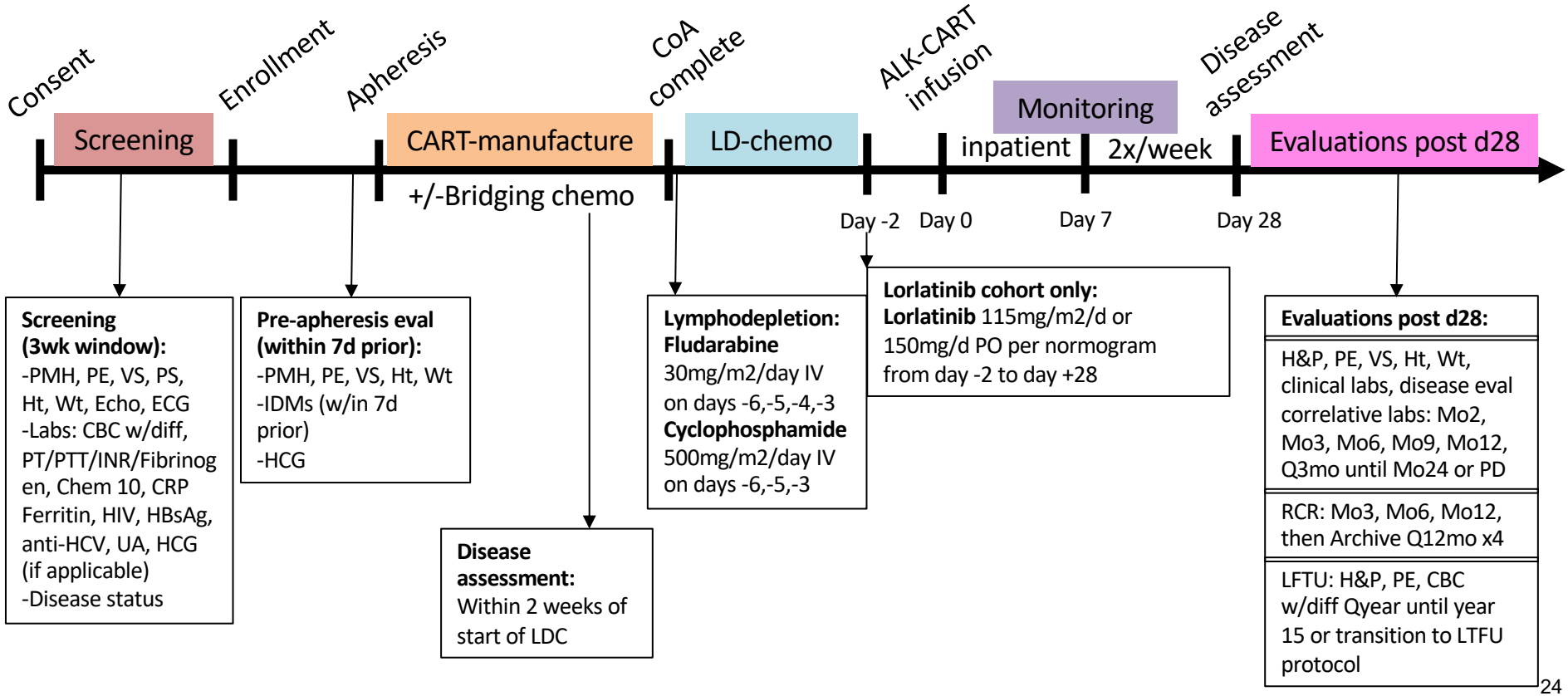
ALK-CART Phase I/II trial Objectives

- Phase 1 Primary Objectives
  - To identify the MTD of autologous ALK-CART cells as measured by the occurrence of DLTs in pediatric patients with r/r HR NBL
  - To identify the safety and tolerability of autologous ALK-CART cells at the MTD and in combination with Lorlatinib 115mg/m2/dose (<18y) or 150mg PO QD (>18y)
- Phase 2 Primary Objectives
  - To estimate the CR and PR rates as per revised International Neuroblastoma Criteria (INRC) in pediatric patients receiving autologous ALK-CART cells +/- Lorlatinib
- Secondary Objectives
  - To estimate Progression free survival (PFS) and Overall Survival (OS)
  - To measure subject reported symptoms
  - Evaluate the biologic activity of ALK-CART cells with detailed correlative studies and correlate these with responses

ALK-CART Phase I/II trial Key Eligibility

- >/= 12months and <30 years
- Histologically confirmed diagnosis of neuroblastoma that is r/r
- HR NBL according to COG risk stratification (LR/IR risk pts who were reclassificed are eligible)
- Histologic confirmation of ALK expression is NOT required
- Recovery/washout from prior therapies (no limit on prior treatment regimens)
- Adequate organ function
- No uncontrolled CNS metastasis (treated and stable for >/= 8 weeks eligible)
- Additional standard eligibility criteria

ALK-CART Phase I/II Clinical Trial Schema



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- **Sarah Nikiforow**
- **Felicia Ciuculescu**
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