MRO . ART
MODERN RADIATION ONCOLOGY
35° RESIDENTIAL COURSE

Emerging Targets in Systemic Therapy: Smarter Drugs, Broader Access?

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GART
Advanced Radiation Therapy

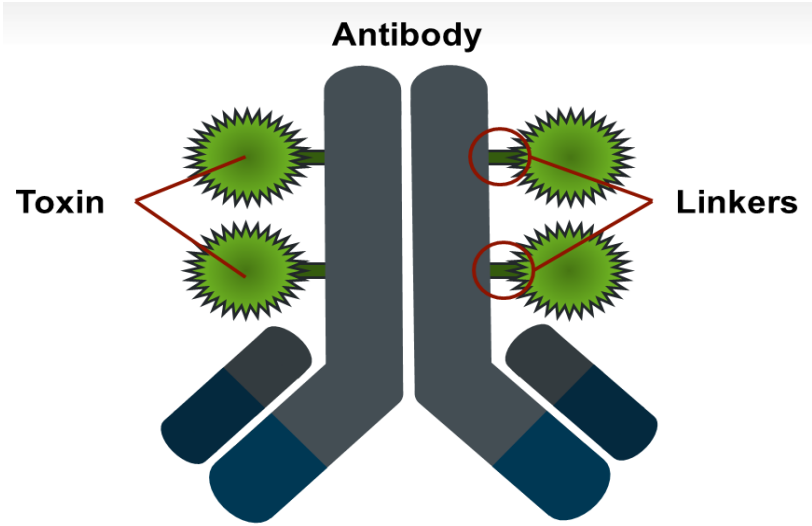
The scenario

- 95% of cancer drugs tested in clinical trials never get approved
  - Because they are too toxic and/or ineffective (at least better than existing)

- only 14% of cancer patients in the United States are treated with precision medicine, and only 7% benefit
  - the main reason for the low application of precision medicine is that most cancers are driven by genetic alterations for which we currently have no drugs

Antibody-Drug Conjugate (ADC)

- Selective delivery of cytoxic drugs via an antibody
- Enhance antibody activity
- Reduces systemic toxicity



The antibody (carrier)

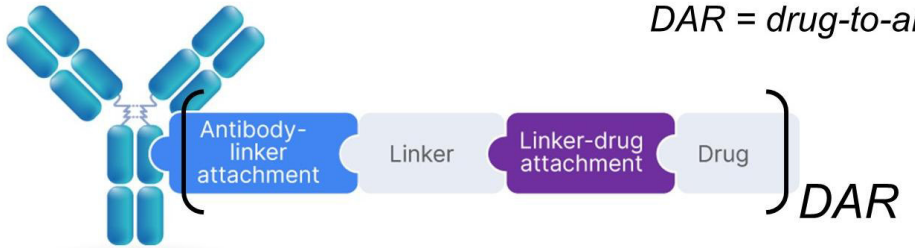
- Humanized IgG antibody
- Directed against a tumor-specific antigen

The linker (key)

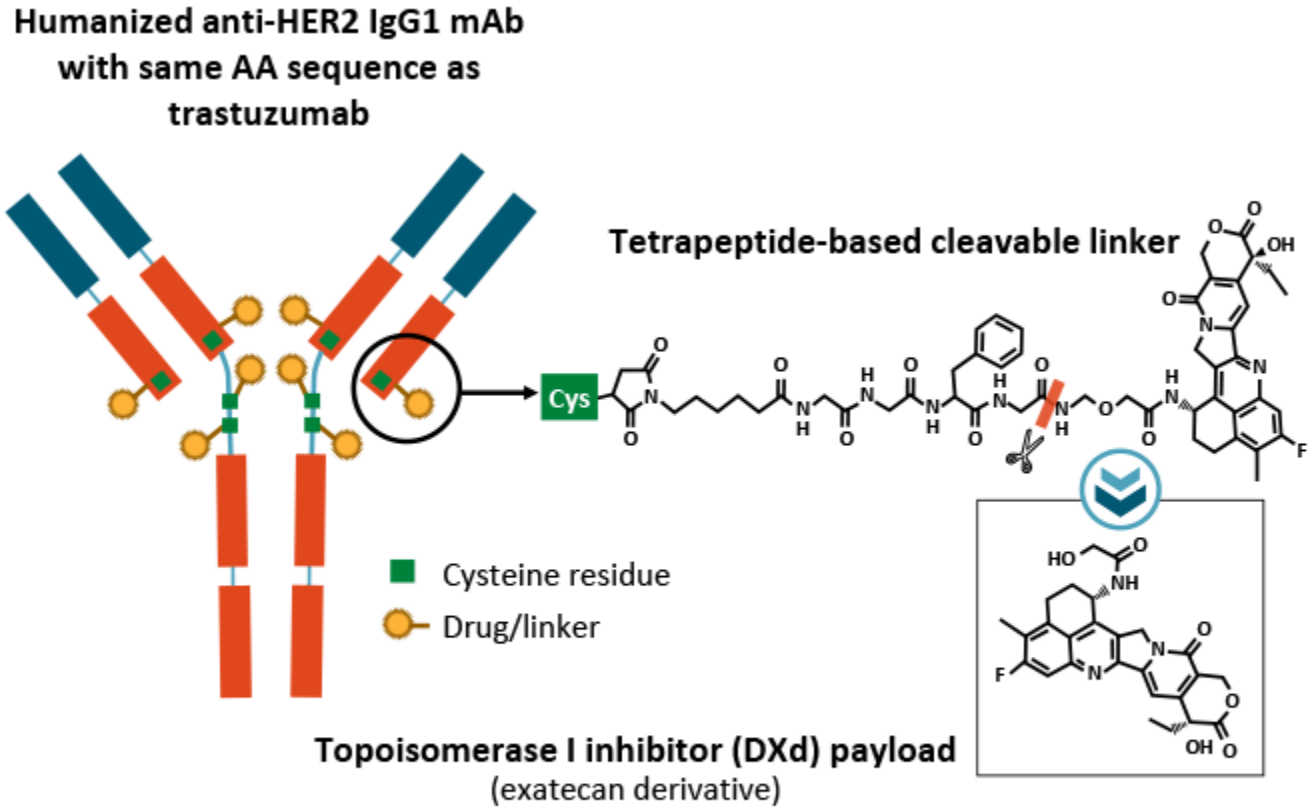
The poison

- Auristatin derivatives (MMAE, MMAF)
- Maytansine derivatives (DM1, DM4)
- Calicheamicin derivatives
- Highly potent cytotoxicity
- IC_{50} at a nM level

DAR = drug-to-antibody ratio



HER2-targeted ADC: Trastuzumab Deruxtecan



- High drug:antibody ratio: ~ 8
- Stable linker-payload
- Tumor-selectable cleavable linker
- High potency, membrane-permeable payload with short systemic half-life
- Bystander killing effect

Nakada. Chem Pharm Bull (Tokyo). 2019;67:173. Trail. Pharmacol Ther. 2018;181:126. Ogitani. Cancer Sci. 2016;107:1039.

Slide credit: clinicaloptions.com

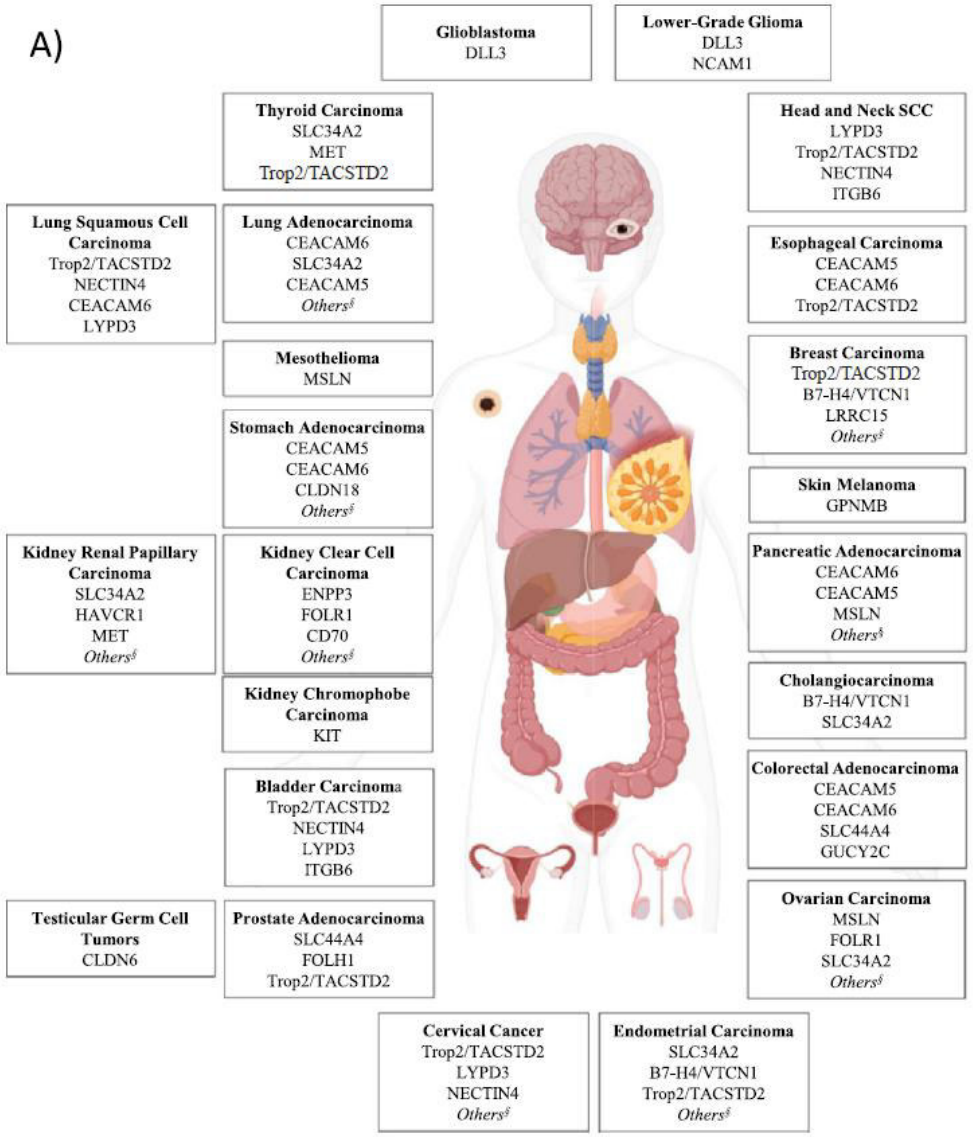
ADCs Revolution: approvals in different cancers

More than 100 ADCs in clinical development across tumor types

Antibody Drug Conjugate	Indication	Company
Trastuzumab deruxtecan	Breast Cancer	Astra Zeneca/Daiichi
Sacituzumab govitecan	Breast Ca, Urothelial Ca	Gilead
Mirvetuximab soravtansine	Ovarian cancer	Immunogen
Tisotumab vedotin	Cervical cancer	Seagen (Pfizer)
Enfortumab vedotin	Urothelial Ca	Seagen (Pfizer)
Trastuzumab DM1	Breast Cancer	Genentech
Loncastuximab tesirine	Lymphoma	ADC Therapeutics
Belantamab mafodotin	Multiple Myeloma	GSK
Polatuzumab vedotin	Lymphoma	Genentech
Inotuzumab ozogamicin	Leukemia	Pfizer
Moxetumomab pasudotox	Leukemia	Astra Zeneca
Brentuximab vedotin	Lymphoma	Seagen (Pfizer)
Gemtuzumab ozogamicin	Leukemia	Pfizer

Ongoing challenges include biomarker development, rational combinations, systematic investigation of components related to ADC mechanism, toxicity management and community education.

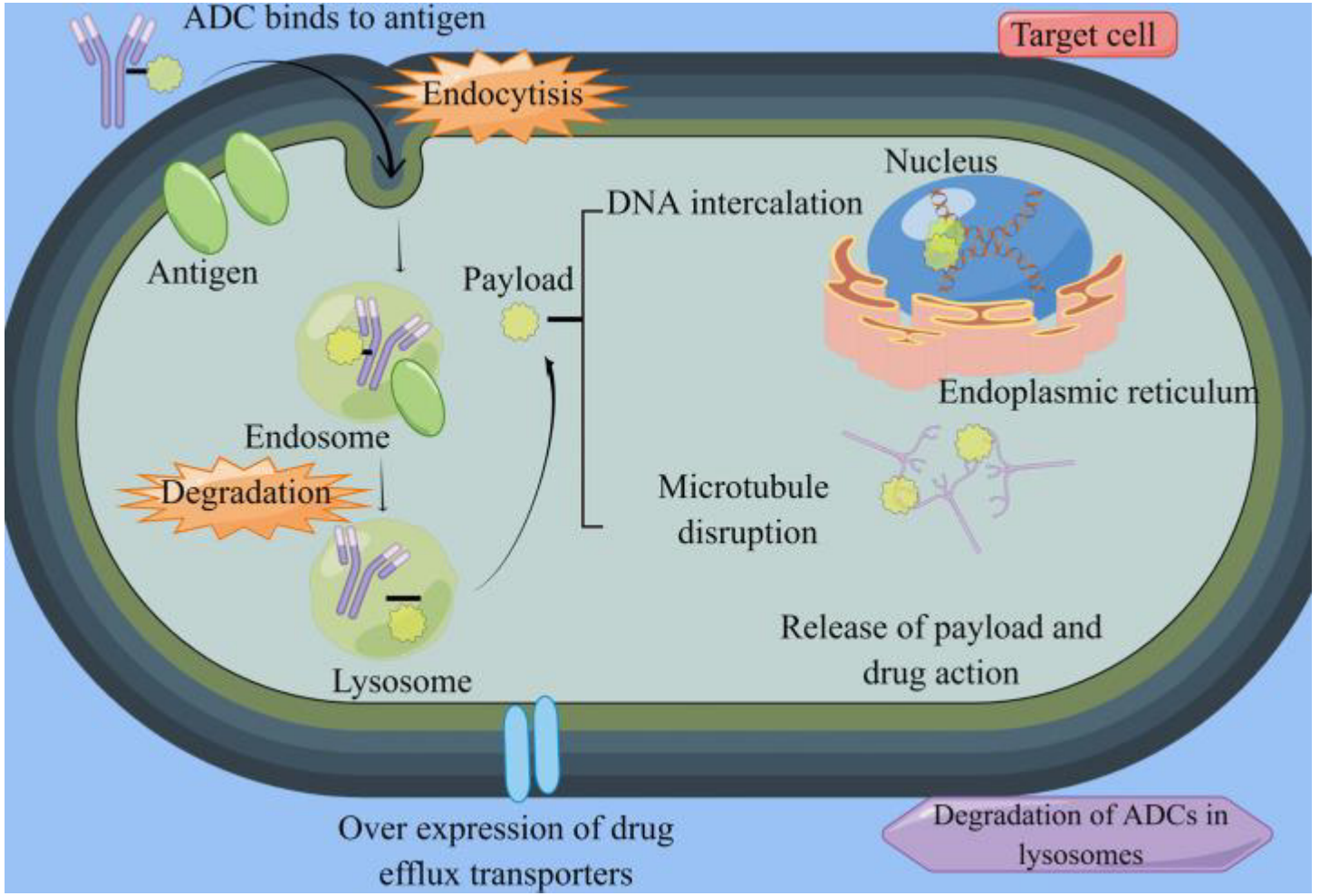
Targets and payloads of ADC under development



ADC CATEGORY [CYTOTOXIN]	KEY PARAMETERS	NUMBER OF ADCS IN DEVELOPMENT
AURISTATINS	• Monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF) • High potency, water solubility, stability and suitability for stable linkers	32
MAYTANSINOIDS	• Second largest class of ADCs in clinical trials are those based on maytansinoids (DM1 and DM4) with four different linkers. • Maytansine binds to the same site on tubulin as the vinca alkaloids, with similar *in vitro* inhibition constants, but is a more-potent cytotoxin.	25
TUBULYSINS	• Tubulysins are antimitotic peptides originally isolated from myxobacteria. • Tubulysins inhibit microtubule polymerization during mitosis to induce cell death and may bypass the efflux pumps for DM1. • A phase I trial to assess the safety and preliminary efficacy of a tubulysin is underway in patients who are refractory to or ineligible for current HER2-targeted therapies.[2]	1
CALICHEAMICIN	• Calicheamicin is a highly potent enediyne antitumor antibiotic originally isolated from the actinomycete Micromonospora echinospora. • A calicheamicin-containing ADC that is directed against ephrin A4, has recently entered phase I trials in TNBC.[3]	5
DUOCARMYCINS	• Duocarmycins are DNA minor groove-alkylating agents. • A conjugate of a human anti-CD70 antibody and a duocarmycin analogue was investigated in a phase I clinical trial in patients with advanced clear-cell RCC and B-NHL and was shown to be well tolerated at doses of up to 8 mg per kg.[4]	2
PYRROLO-BENZODIAZEPINE (PBD)	• PBDs are based on naturally occurring antitumor antibiotics that bind to the DNA minor groove in a sequencespecific manner. • PBD dimers have picomolar activity against many human tumor cell lines. • They are generally not substrates for MDR1 and thus retain activity in MDR1+ tumors and in tumors that are refractory to gemtuzumab ozogamicin treatment. • At least ten ADCs that are based on Spirogen's PBD dimer warheads have entered clinical trials, which makes them the third most prominent class of payloads after auristatins and maytansinoids.[1] • Five PBD-based ADCs are homogeneous third-generation ADCs that are based on engineered cysteine mAbs (EC-mAbs) and are directed against CD33, CD70, CD19, CD123 (also known as IL-3Rα) and CD352, respectively.[5,6]	11
DOXORUBICIN	• Doxorubicin is an actinomycete-derived antimitotic anticancer agent that is routinely used in the clinic. • Milatuzumab doxorubicin is an ADC that is directed against CD74, which is an antigen that is associated with haematological tumors, and is currently being investigated in phase II trials in chronic lymphocytic leukemia (CLL) and NHL.[7]	1

Bosi et al., Eur J Cancer 195 (2023)

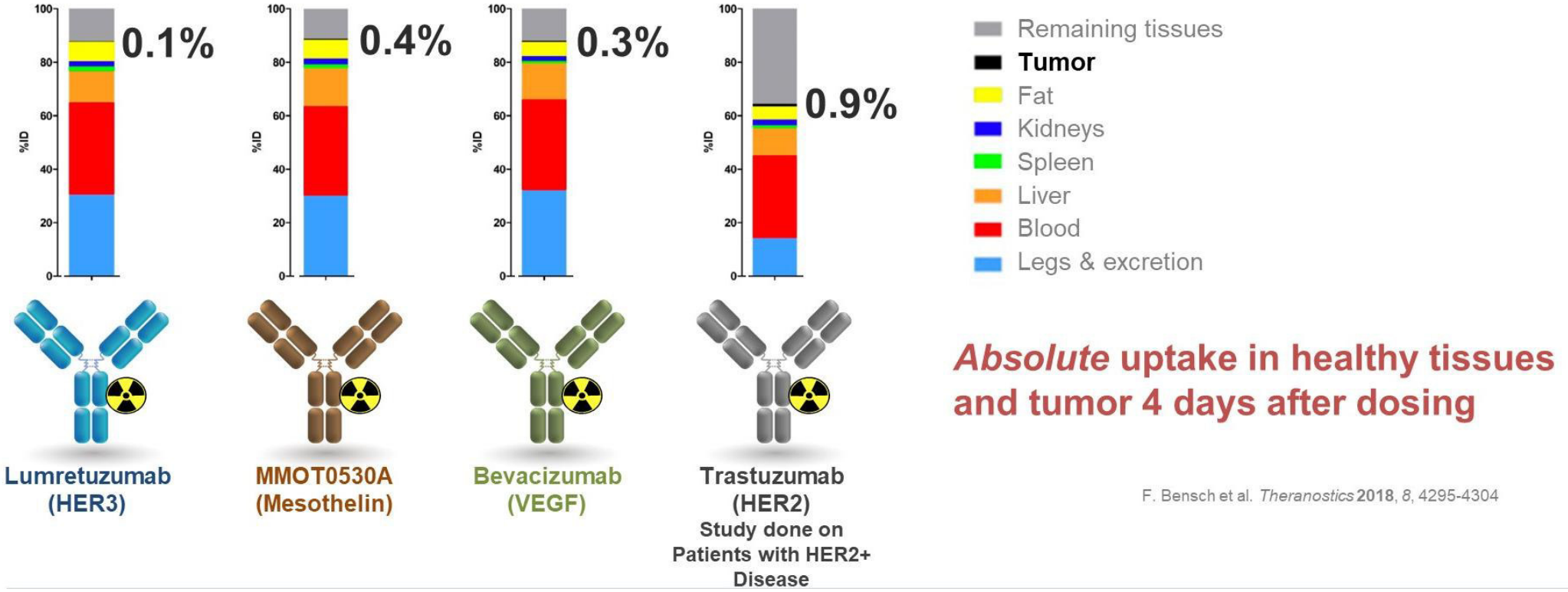
ADC Mechanism of action



A small fraction of ADC enters the tumor

Radiolabeled Antibodies can reveal the fate of ADC Therapeutics

Irrespective of the target, radiolabeled antibodies show high normal tissue distribution and <1% tumor uptake in humans



Absolute **uptake in healthy tissues and tumor 4 days after dosing**

F. Bensch et al. *Theranostics* **2018**, *8*, 4295-4304

ADCs activity is not always related to target expression:

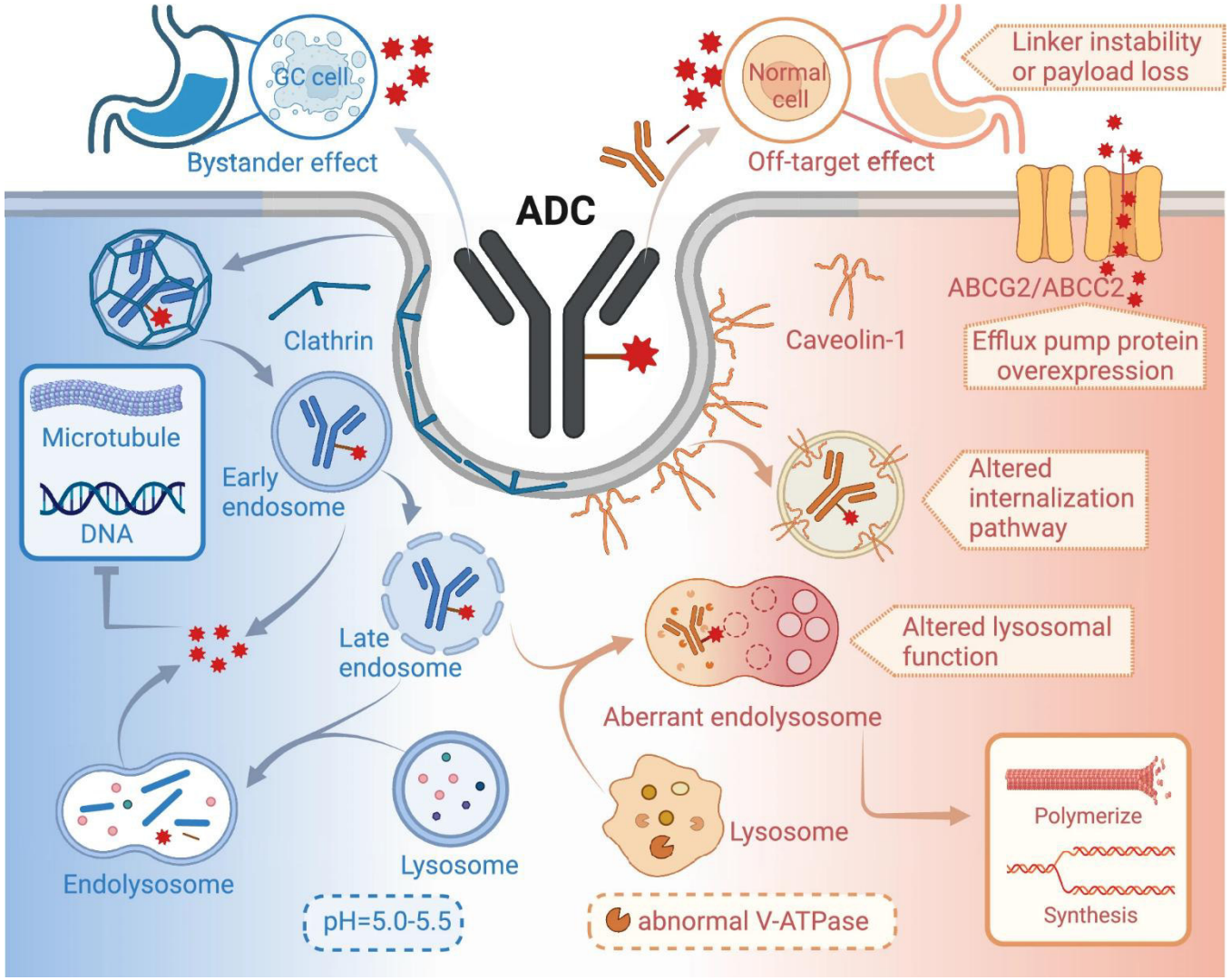
In HER2-negative mBC, Sacituzumab govitecan benefit is maintained regardless of Trop-2 expression

Tisotumab vedotin activity is not associated with Tissue Factor (TF) expression

NECTIN4 amplification predicts enfortumab vedotin responses and long-term efficacy

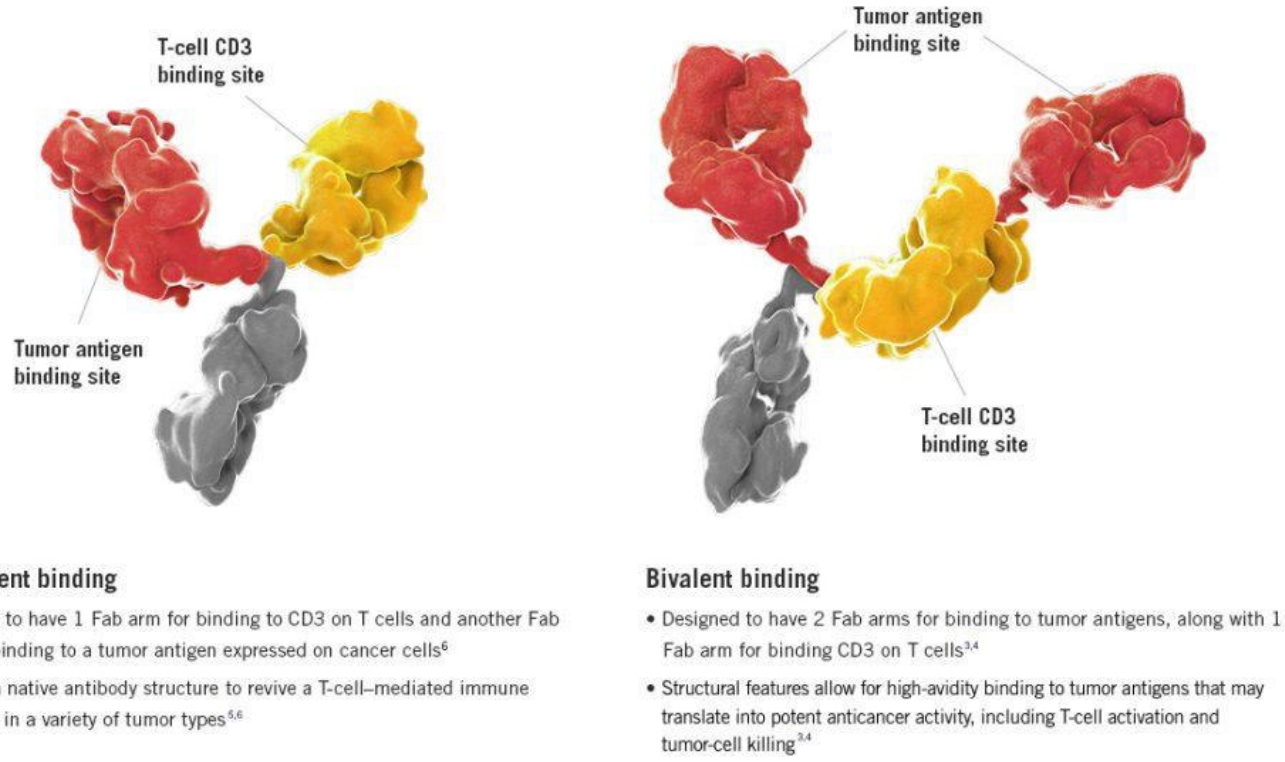
looking for biomarkers

Mechanisms of Resistance to ADCs



Mechanism of action **Relative mechanism of Resistance**

Bispecific antibodies T cell engagers



Monovalent binding

- Designed to have 1 Fab arm for binding to CD3 on T cells and another Fab arm for binding to a tumor antigen expressed on cancer cells[6]
- Utilizes a native antibody structure to revive a T-cell–mediated immune response in a variety of tumor types[5,6]

Bivalent binding

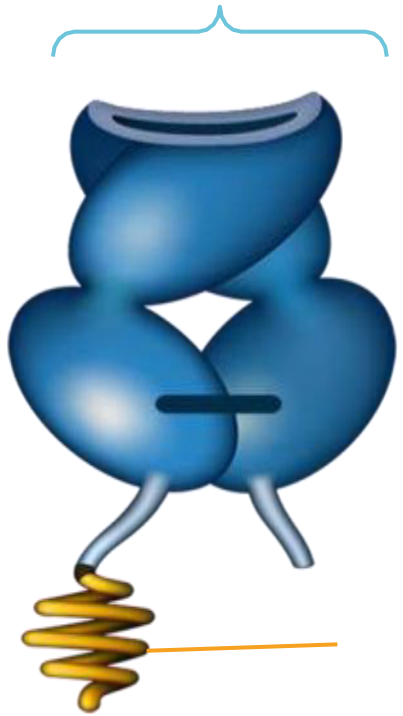
- Designed to have 2 Fab arms for binding to tumor antigens, along with 1 Fab arm for binding CD3 on T cells[3,4]
- Structural features allow for high-avidity binding to tumor antigens that may translate into potent anticancer activity, including T-cell activation and tumor-cell killing[3,4]

Simultaneous binding of a T-cell bispecific antibody to CD3 on T cells and a tumor cell surface antigen, may result in the formation of an immune synapse, a junction formation between the T cell and tumor cell, and subsequent downstream signaling that may lead to T-cell–mediated tumor killing through:

- T-cell activation and secretion of cytotoxic granules
- Secretion of cytokines/chemokines that generate a pro-inflammatory tumor microenvironment and recruit additional T cells
- T-cell proliferation and expansion at the tumor site

Tebentafusp has a high specificity and affinity for HLA-A*0201-gp100$_{280-288}$

TCR affinity is increased from µM to pM[1,4]



- Tebentafusp is illustrative of the ImmTAC® platform with its bispecific effector and targeting ends[1-4]
- The targeting end is an affinity-enhanced soluble TCR[1]
  - High target affinity facilitates detection of low cell surface levels of HLA-A*0201-gp100$_{280-288}$ [1-4]
  - Targets HLA-A*0201-gp100$_{280-288}$, with high specificity and low cross reactivity[1,2,5]
- The effector end (anti-CD3) binds to and activates T cells via CD3[1-4]

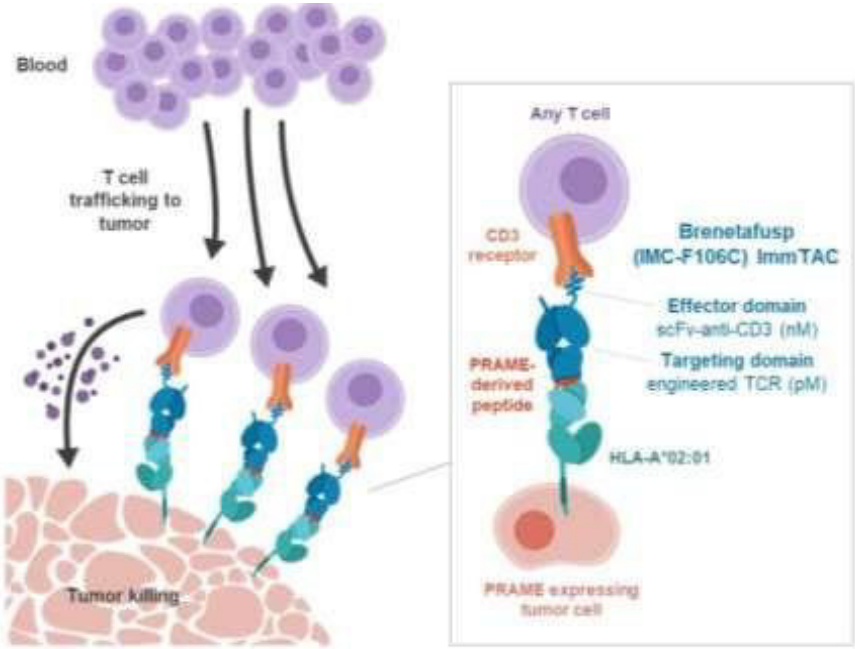
*Of 531 cancers investigated by GENVESTIGATOR, melanomas comprised the top ten gp100-expressors.[5]
scFv: single-chain variable fragment; $T_{½}$: Half-life; TCR: T cell receptor.

1. Damato BE, et al. *Cancers (Basel)* 2019;11:971–87; 2. IMCgp100-202 Protocol v5.0. Data on file; 3. Liddy N, et al. *Nat Med* 2012;18:980–7; 4. Middleton MR, et al. *Clin Cancer Res* 2020;26(22):5869–78; 5. Bossi G, et al. *Cancer Immunol Immunother* 2014;63:437–48.

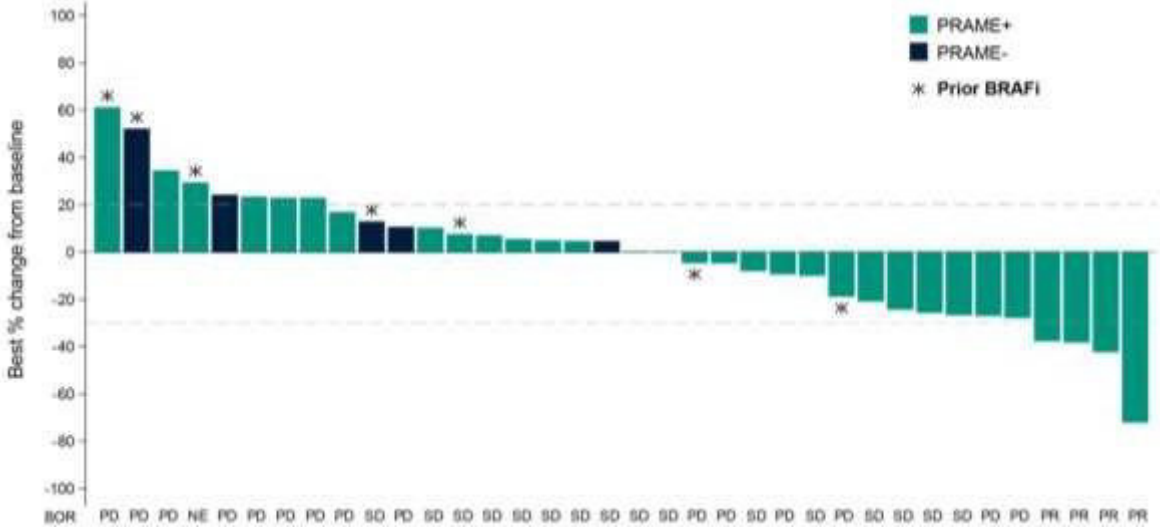
2024 ASCO ANNUAL MEETING

Phase 1 safety and efficacy of brenetafusp (IMC-F106C), a PRAME × CD3 ImmTAC bispecific, in post-checkpoint cutaneous melanoma (CM)

- TCR bispecific ImmTAC molecules redirect polyclonal T cells to target cancer cells by recognizing intra-/extra-cellular cancer proteins
- ImmTAC platform validated by tebentafusp (gp100 × CD3) with OS (HR 0.51) and PFS benefit (HR 0.73) in mUM[2]
- ImmTAC tolerability with immune checkpoints demonstrated with tebentafusp in cutaneous melanoma[2]
- PRAME is broadly expressed in several tumor types, including ~90% cutaneous melanoma (CM), with minimal normal tissue expression[1]

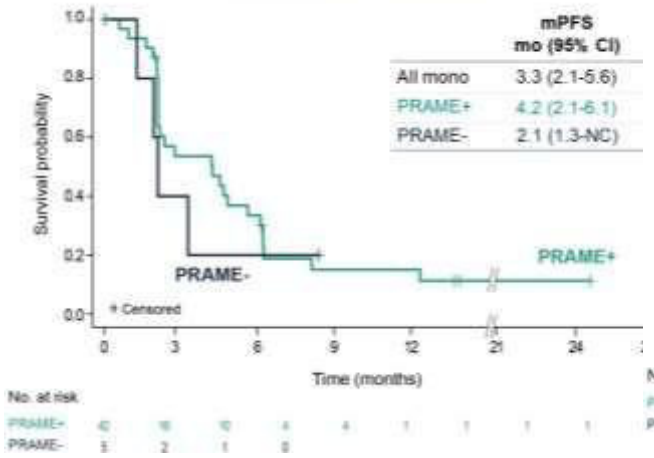


Tumor reduction observed only in PRAME+ pts

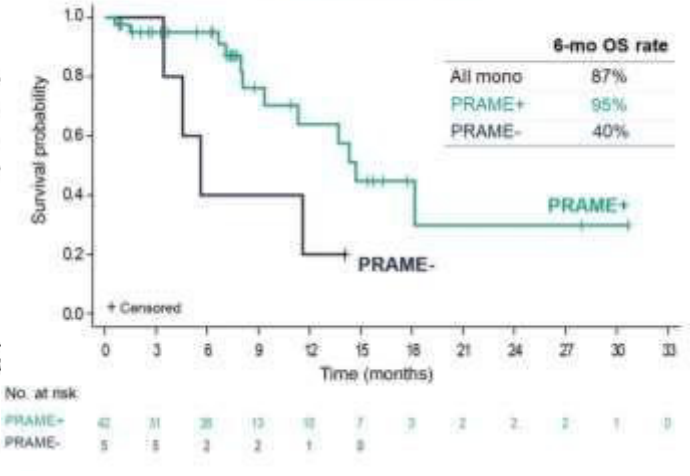


Promising initial PFS and OS, enriched in PRAME+ pts

Progression free survival

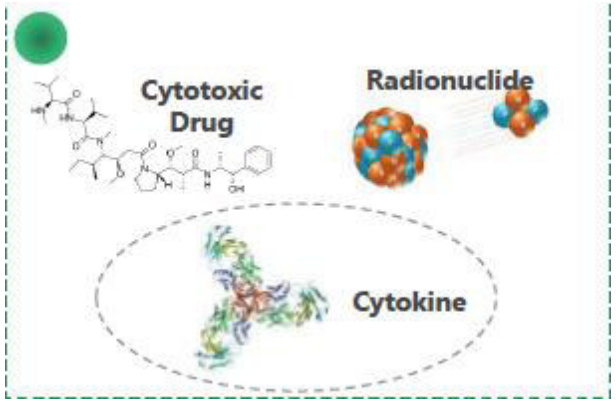


Overall survival



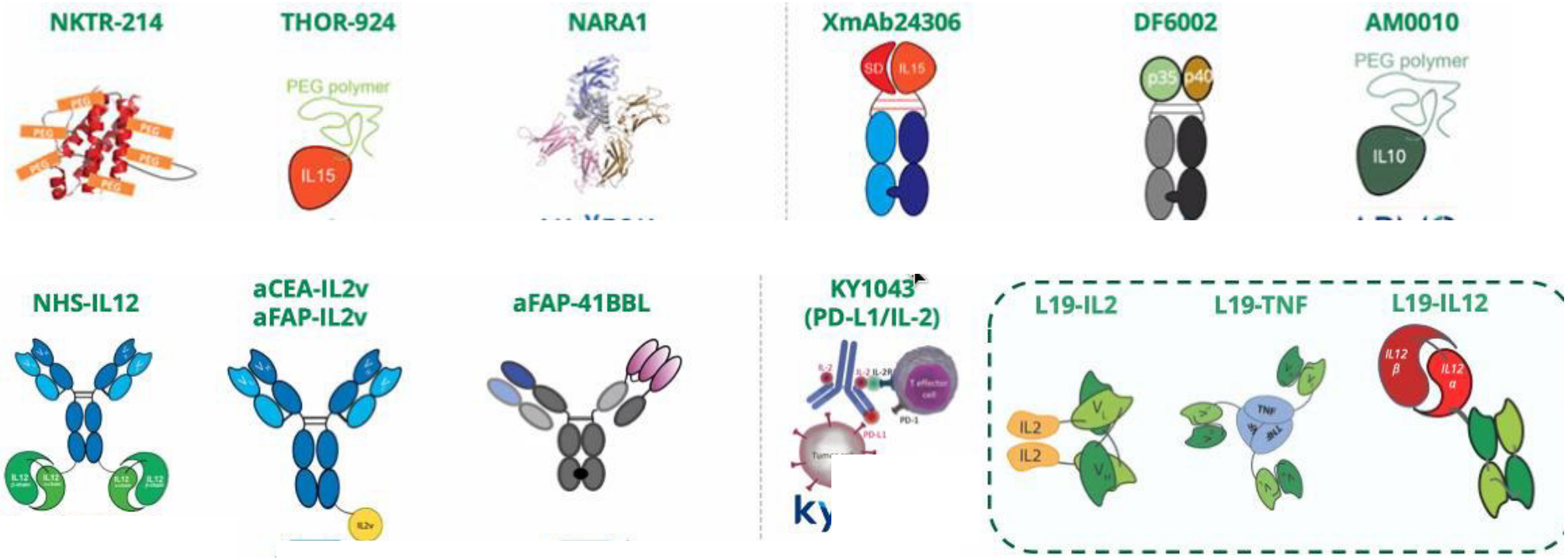
Adapted from Hamid, ASCO 2024

New engeenered antibodies with different payloads



CYTOKINES	**IL1b** (Hess, 2014) **IL2** (Carnemolla, 2002) **IL3** (Schmid, 2018) **IL4** (Hemmerle, 2014) **IL5** (unpublished) **IL6** (Hess, 2014) **IL7** (Pasche, 2012) **IL9** (Venetz, 2015) **IL10** (Trachsel, 2007) **IL12** (Halin, 2002) **4-1BBL** (Mock, 2020)	**IL13** (Hess, 2015) **IL15** (Kaspar, 2007) **IL17** (Pasche, 2012) **IL18** (unpublished) **IL22** (Bootz, 2016) **IFNa** (Frey, 2010) **IFNb** (unpublished) **IFNg** (Ebbinghaus, 2005) **TNF** (Borsi, 2003) **TRAIL** (Hemmerle, 2014)	**TRAILtrunc** (Hemmerle, 2014) **CD40L** (Hemmerle, 2014) **FasL** (Hemmerle, 2014) **LiGHT** (Hemmerle, 2014) **VEGI** (Hemmerle, 2014) **VEGItrunc** (Hemmerle, 2014) **LT-a** (Hemmerle, 2014) **LT-b** (Hemmerle, 2014) **LT-a1b2** (Hemmerle, 2014) **G-CSF** (Schmid, 2018) **GM-CSF** (Kaspar, 2007)
CHEMOKINES	**CCL5** (Hess, 2014) **CCL17** (Hess, 2014) **CCL19** (Hess, 2014)	**CCL20** (Hess, 2014) **CCL21** (Hess, 2014) **CXCL4** (Hess, 2014)	**CXCL9** (Hess, 2014) **CXCL10** (Hess, 2014) **CXCL11** (Hess, 2014) **ITIP** (Hess, 2014)
OTHER PAYLOADS	**B7.2** (Hemmerle, 2012) **tTF** (Nilsson, 2001)	**TNFR** (Schwager, 2009) **VEGF-A^{120}** (Halin, 2002)	**VEGF-A^{164}** (Halin, 2002) **VEGF-C** (Schwager, 2018) **other undisclosed payloads**

Engeenered cytokine therapeutics : new frontier and large investments



Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer

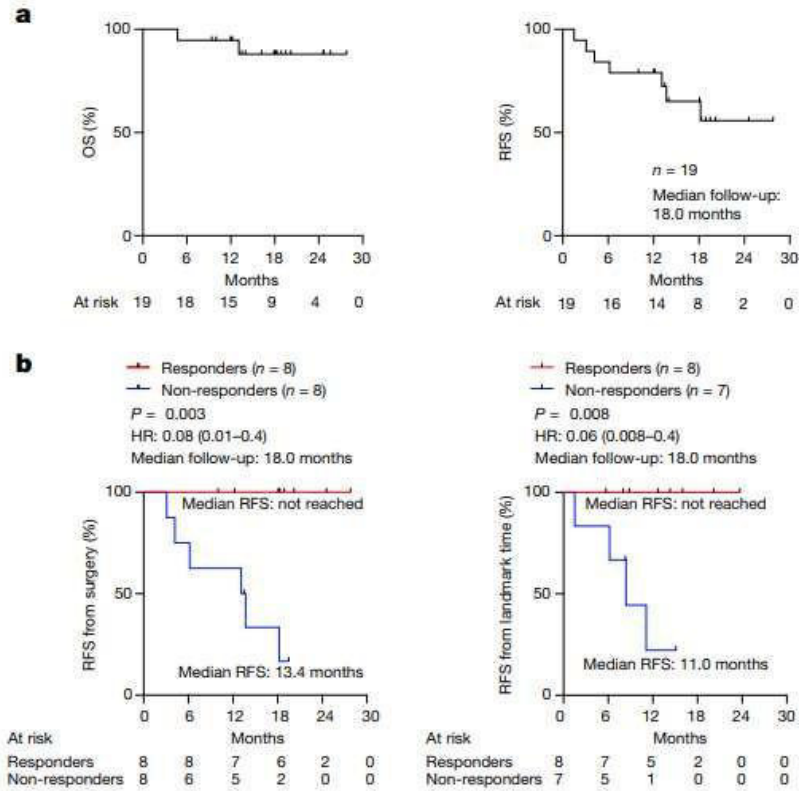


Fig. 3 | mRNA vaccine response correlates with delayed PDAC recurrence. **a**, OS and RFS in $n = 19$ patients in the safety-evaluable cohort. **b**, RFS from surgery and from landmark time (date of the last vaccine priming dose) stratified by vaccine response in patients in the biomarker-evaluable cohort. *n* indicates individual patients. HR indicates hazard ratio with 95% CI. *P* values calculated using two-tailed log-rank test.

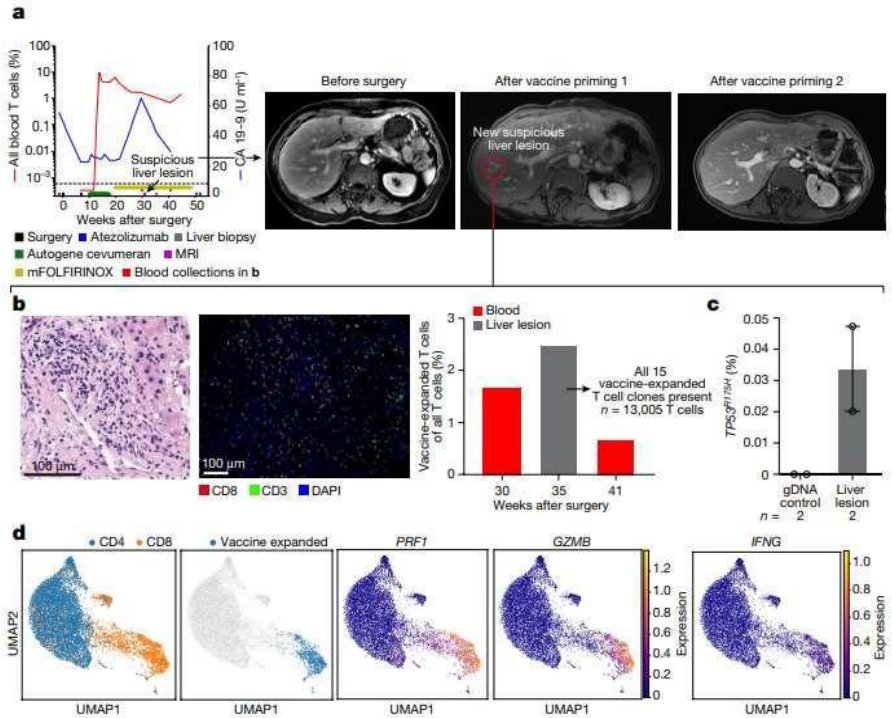
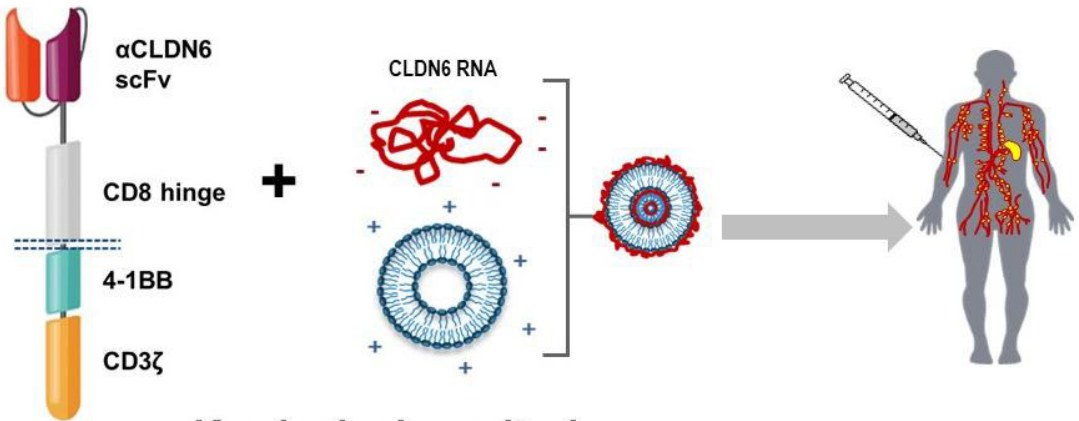


Fig. 4 | Vaccine-expanded T cells can infiltrate a micrometastasis. Clinical and immunological snapshot of a disappearing intrahepatic lymphoid aggregate after vaccination in a patient who responded to the vaccine. **a**, Serial percentage of vaccine-expanded T cells in blood analysed using CloneTrack and serum CA19-9 (left), and abdominal MRI (right) before and after vaccination. **b**, Haematoxylin and eosin staining (left), multiplexed immunofluorescence (middle) and percentage of vaccine-expanded T cells measured using CloneTrack (right, grey bar) in a new liver lesion that developed after vaccination detected by MRI as in **a**. All 15 vaccine-expanded T cell clones (**a**, red line) were present in liver lesion (right, grey bar). **c**, Percentage of mutant *TP53*^{R175H} reads by digital droplet PCR in the liver lesion. The bar indicates the median, the error bars are the s.e.m. **d**, Uniform manifold approximation and projection (UMAP) plots of single-cell phenotypes of all blood T cells (left) and vaccine-expanded clones (middle), with effector markers (right). *n* indicates the number of T cells detected in liver lesion (**b**) or technical replicates (**c**). Data represent analyses of a single patient.

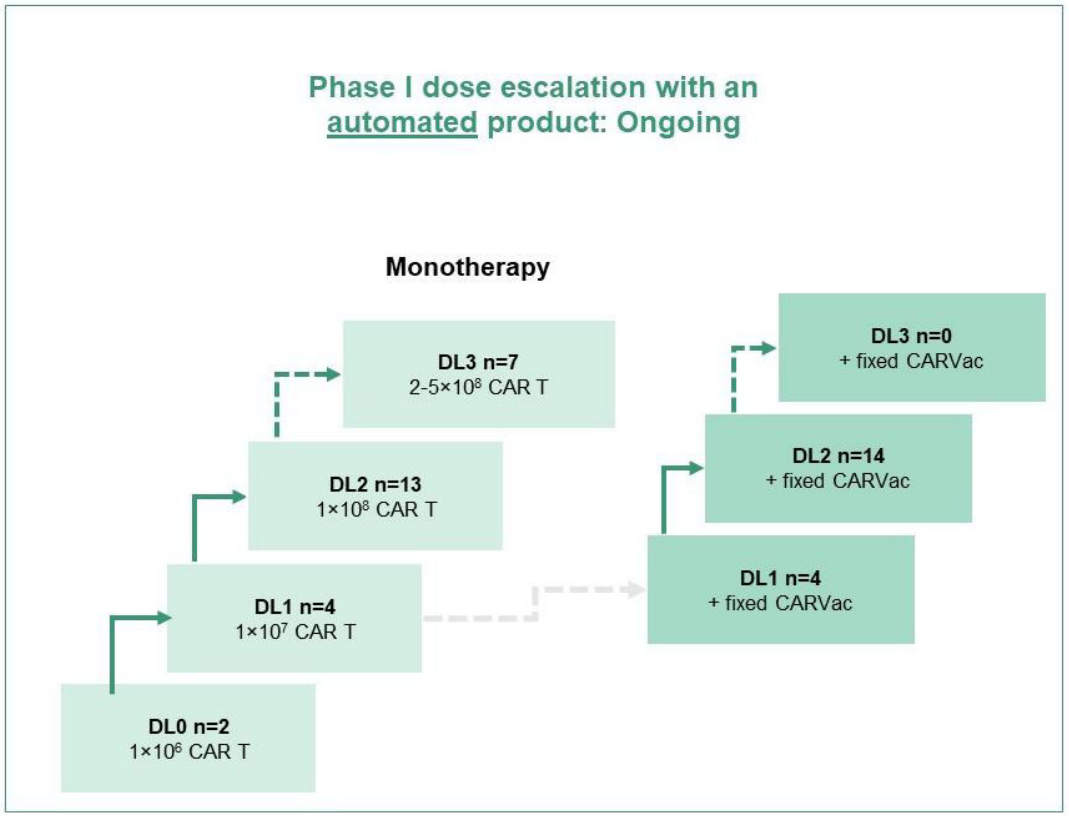
Combining CAR-T with Amplifying RNA Vaccine

Phase I Study of CLDN6 CAR-T cells + a CLDN6-encoding CAR-T cell-Amplifying RNA Vaccine (CARVac): BNT211-01



Key inclusion criteria

- ≥50% tumor cells with 2+/3+ CLDN6 positivity (immunohistochemistry)
- Measurable disease per RECIST v1.1 or elevated tumor marker
- ECOG PS 0–1

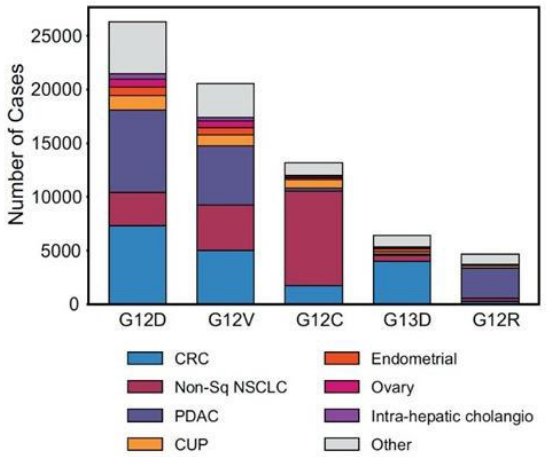


NCT04503278
Haanen et al. *ESMO 2023*, LBA35
Mackensen et al. *Nat Med*, 2023

NT-112: an armored TCR-T targeting KRAS G12D mutant tumors

KRAS mutation frequency[1]

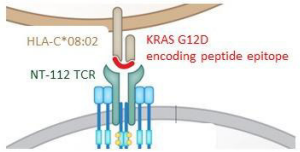
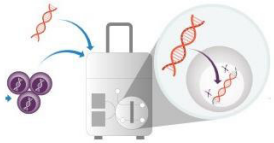
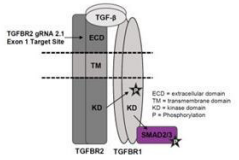
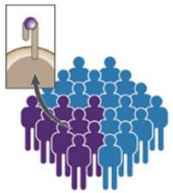
Pancreas	Colorectal	Lung	Endometrial
67%	45%	30%	24%



KRASG12D and HLA-C*08:02 - Approx. 9,000 patients/ year in US + EU[3,4]

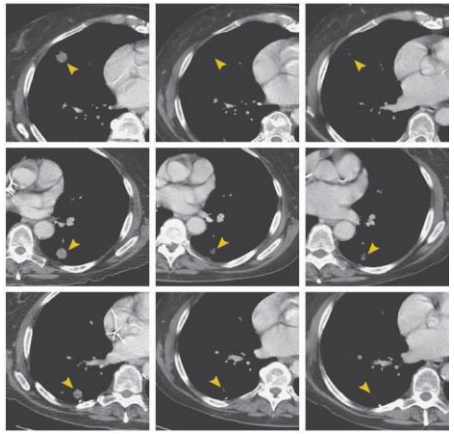
Susan Galbraith, AZ, ASCO, 2024

HLA-C*08.02 restricted T Cell Receptors x2 targeting *KRAS* G12D

Mutation/HLA allele targeted with NT-112	Non-viral nuclease-based manufacturing	Armored through TGFBR2 KO	First Phase I trial initiated in US
			
• TCR directed against **KRAS G12D** • HLA allele: **HLA-C*08:02**	• Site-specific integration of TCR genes in the TCR locus & multiplexed gene knock-out	• Genetic knockout of TGFBR2 in TCR edited T cells to **maintain T cell function in TGF-β rich TME**	• Phase I trial initiated targeting patients with **Pancreas, CRC, Lung, Endometrial and Ovarian** • NCT06218914

Ongoing 6-month Partial Response in metastatic pancreatic cancer[5]

Pre- Treatment Day 85 Day 176



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Neoantigen T-Cell Receptor Gene Therapy in Pancreatic Cancer

Rom Leidner, M.D., Nelson Sanjuan Silva, B.S., Huayu Huang, M.S., David Sprott, B.S., Chunhong Zheng, Ph.D., Yi-Ping Shih, Ph.D., Amy Leung, B.S., Roxanne Payne, M.N., Kim Sutcliffe, B.S.N., Julie Cramer, M.A., Steven A. Rosenberg, M.D., Ph.D., Bernard A. Fox, Ph.D., Walter J. Urba, M.D., Ph.D., and Eric Tran, Ph.D.

KRAS Adoptive cell therapies, vaccines

- anti-RAS-G12Dm TCR HLA A*11:01
- anti-RAS-G12Vm TCR HLA A*11:01
- mRNA-5671/V941 (neopeptides G12C,D,V, G13D) +/- pembrolizumab
- ELI-002 mKRAS G12D, G12R amphi-peptides
- mKRAS peptide, poly-ICLC + ipilimumab, nivolumab
- mKRAS TCR, HLA specific

CRISPR–Cas9 enter into the clinics for immunetherapy

Article

Non-viral precision T cell receptor replacement for personalized cell therapy

https://doi.org/10.1038/s41586-022-05531-1
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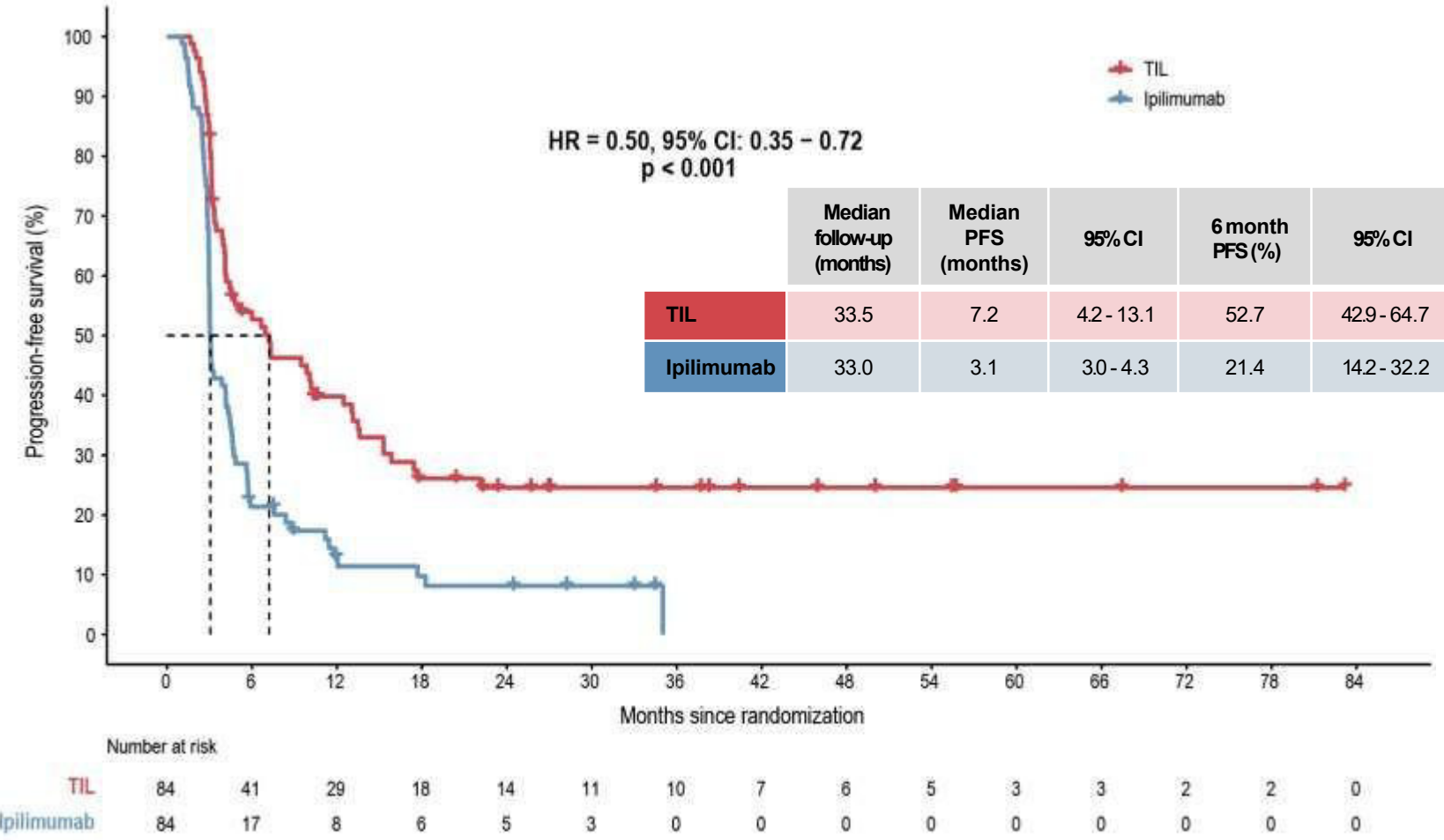
- with the help of CRISPR, genetically redirect T cells to mutant neoantigens.
- The non-viral gene editing designed to knock in and out genes in order to improve T cell function.
- The edits helped circumvent immunosuppression, and so, the T cells could elicit a response despite many antigen encounters.

- A clinical-grade approach based on CRISPR–Cas9 non-viral precision genome-editing to simultaneously knockout the two endogenous TCR genes.
- Also, two chains of a neoantigen-specifc TCR (neoTCR) isolated from circulating T cells of patients were inserted into the TRAC locus, using a personalized library of soluble predicted neoantigen–HLA capture reagents.
- Sixteen patients with different refractory solid cancers received up to three distinct neoTCR transgenic cell products, each expressing a patient-specifc neoTCR; administered in a cell-dose-escalation, first-in-human phase I trial.
- One patient had grade 1 cytokine release syndrome and one patient had grade 3 encephalitis. All participants had the expected side effects from the lymphodepleting chemotherapy.
- 5 patients had SD and the other 11 had a PD.
- neoTCR transgenic T cells were detected in tumour biopsy samples after infusion at frequencies higher than the native TCRs before infusion.

- This study demonstrates the feasibility of isolating and cloning multiple TCRs that recognize mutational neoantigens. Moreover, simultaneous knockout of the endogenous TCR and knock-in of neoTCRs using single-step, non-viral precision genome-editing are achieved.

Treatment with tumor-infiltrating lymphocytes (TIL) versus ipilimumab for advanced melanoma: results from a multicenter, randomized phase 3 trial

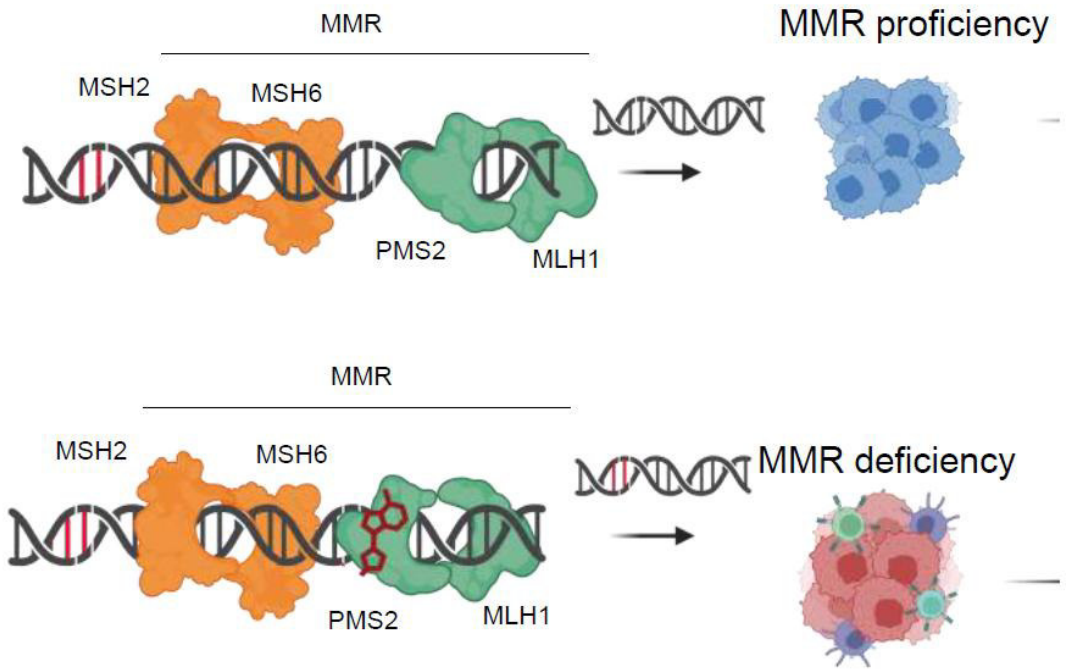
PFS according to RECIST 1.1 in the ITT population

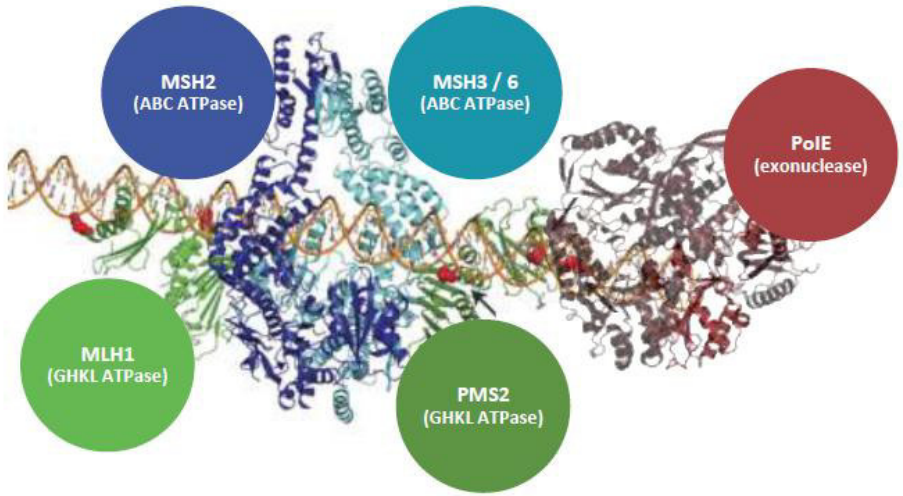


	Median follow-up (months)	Median PFS (months)	95% CI	6 month PFS (%)	95% CI
TIL	33.5	7.2	4.2 - 13.1	52.7	42.9 - 64.7
Ipilimumab	33.0	3.1	3.0 - 4.3	21.4	14.2 - 32.2

John B.A.G. Haanen, ESMO 2022

Can we pharmacologically block MMR?

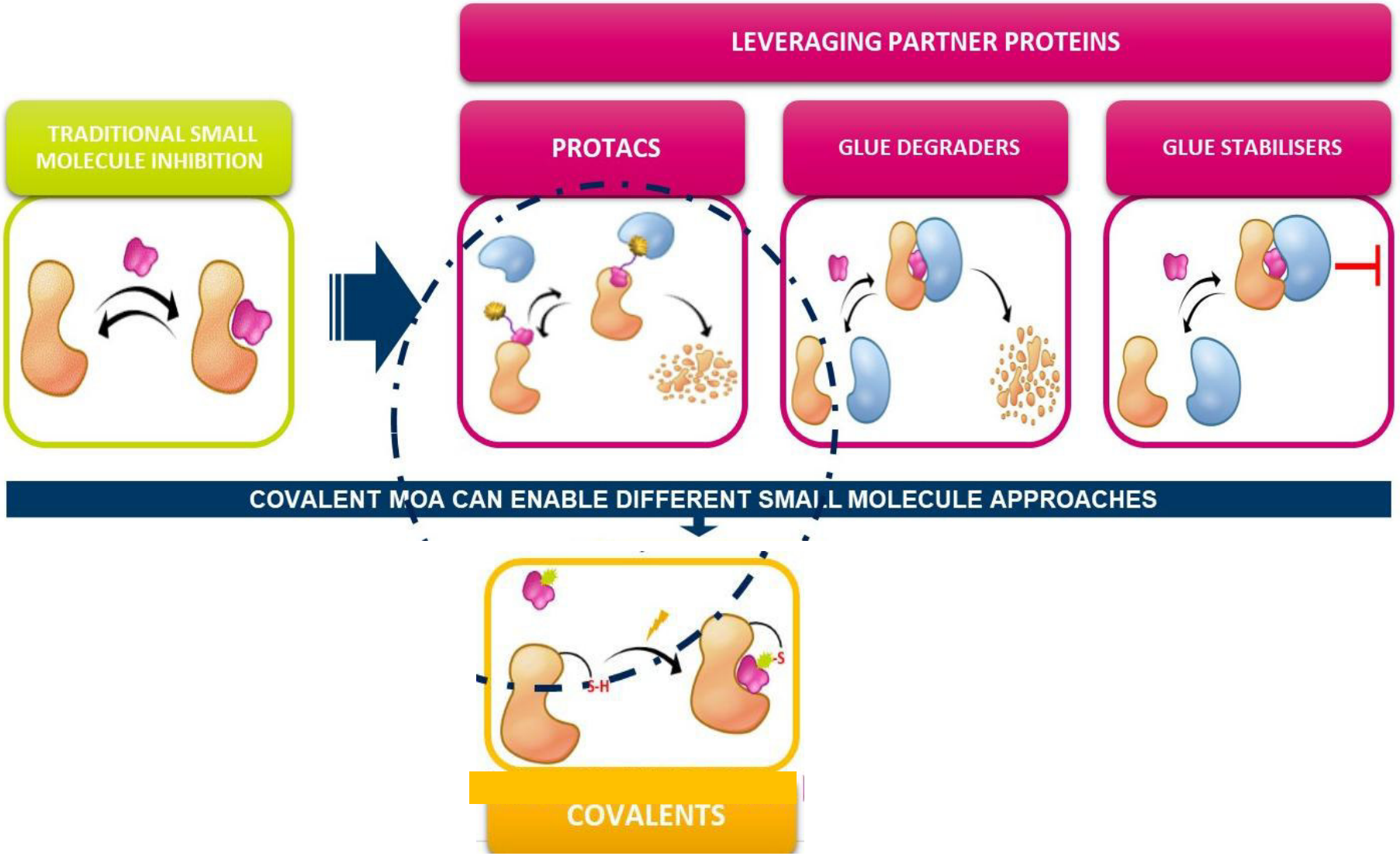




Novel emerging biology suggests MMR controls a variety of signaling nodes related to immunosurveillance

Prof. A. Bardelli

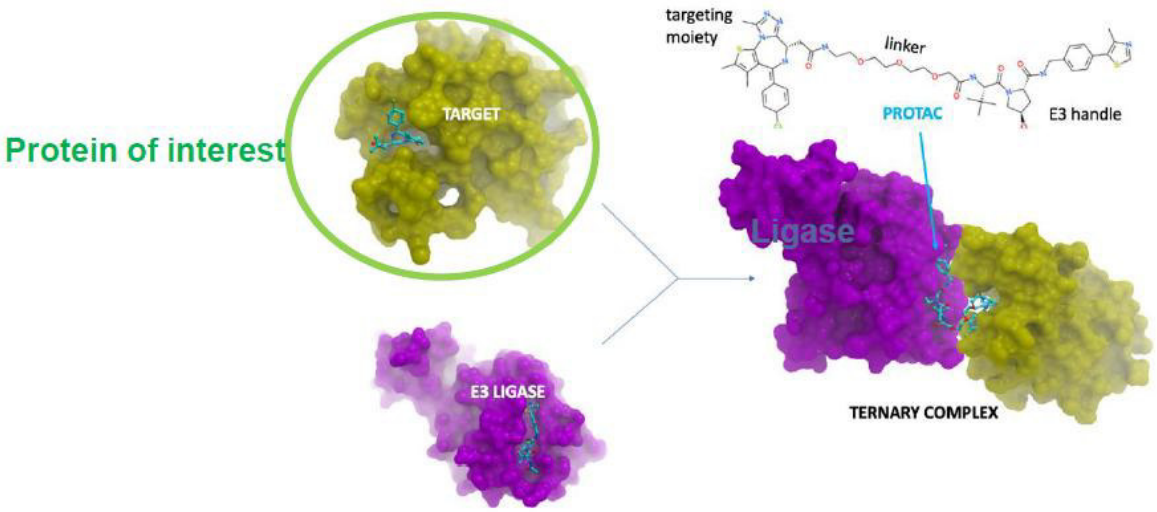
Develop a diversity of small molecules approach



S. Galbraith, A-Z, ASCO 2024

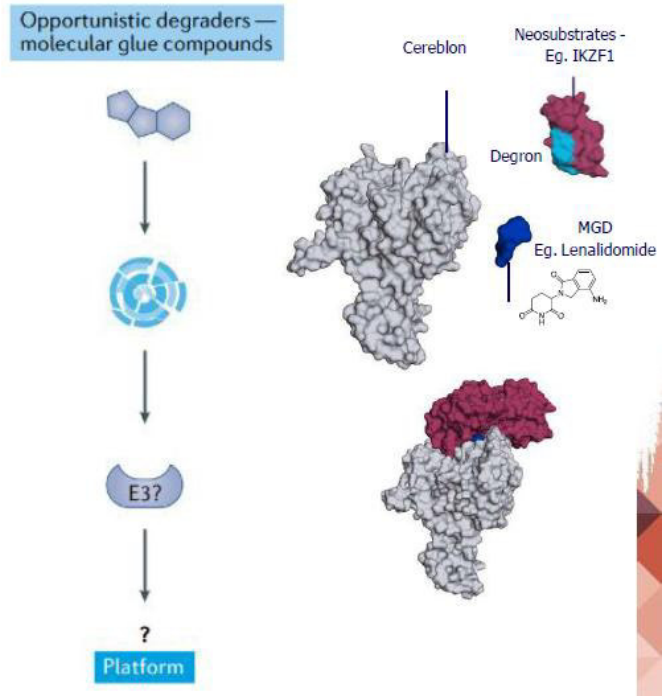
Targeting the Ubiquitine proteasome Complex
Protein degraders

Proteolysis Targeting Chimeras (PROTACs)

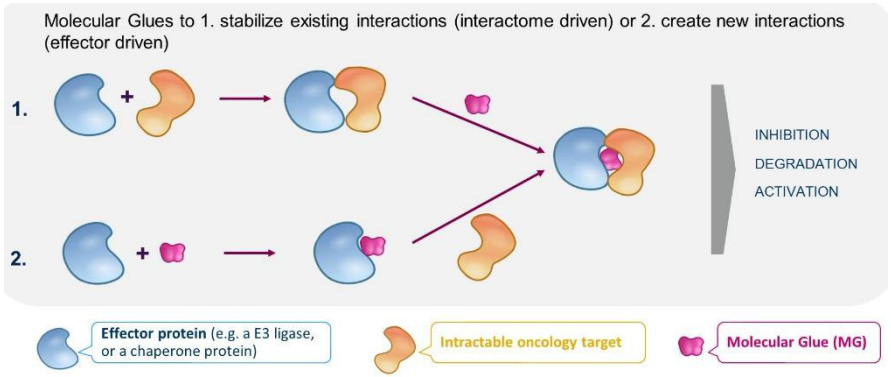


PROTACs chemical structure.

Molecular Glue Degraders



“molecular glue” degraders, tighten and simplify the connection of E3 ligase with target protein for



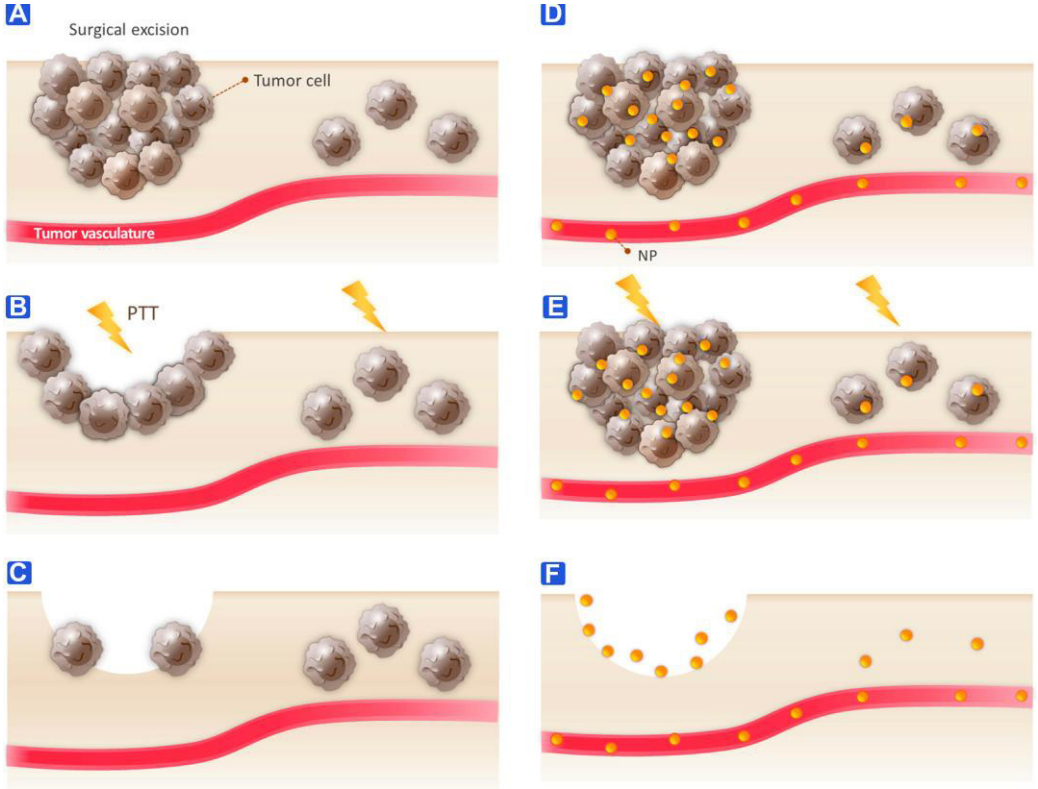
Jordi Rodon, ESMO 2024; Yong K, Eur J Haemat, 2018; Galbraith ASCO 2024

The use of Nanoparticles in Photo thermal therapy (PTT) and immunetherapy

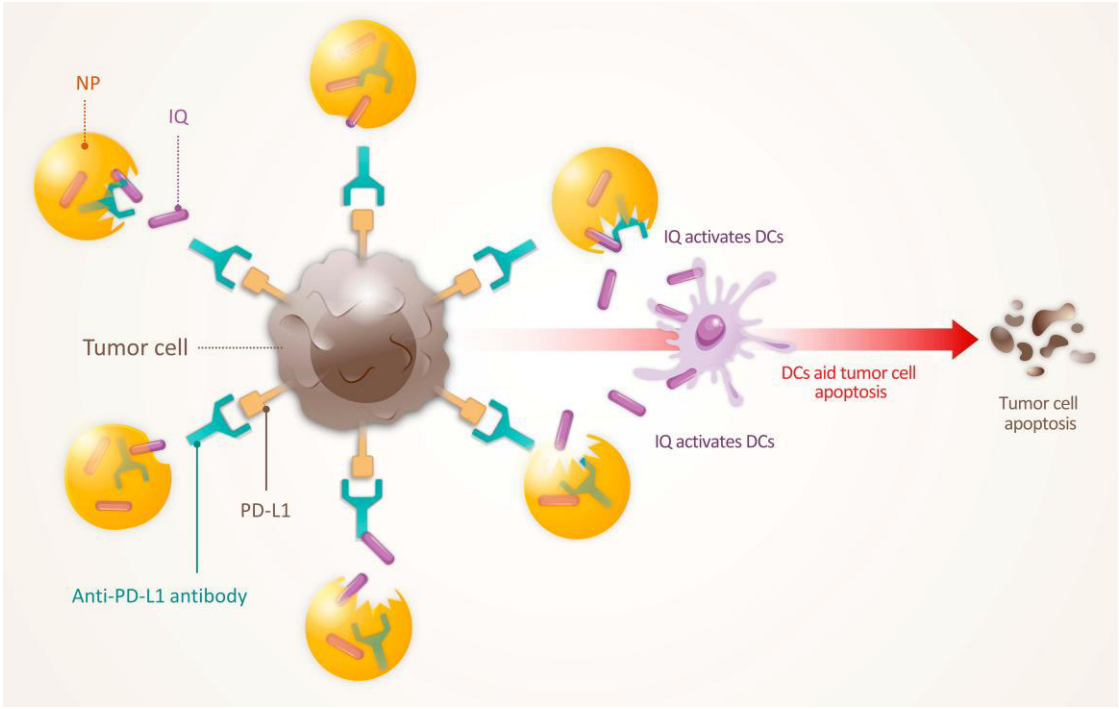
Polydopamine-coated gold NPs are injected in the tumor Vasculature and then activated with PTT

Conventional surgery

NPConventional surgery



Entrapment of Imiquimod (IQ) in polydopamine NPs (PNs) represents adjuvant-loaded NPs (IQ/PNs). With the help of anti-PD-L1 MAbs, IQ/PNs are able to efficiently bind tumor cells overexpressing PD-L1, causing tumor cells apoptosis



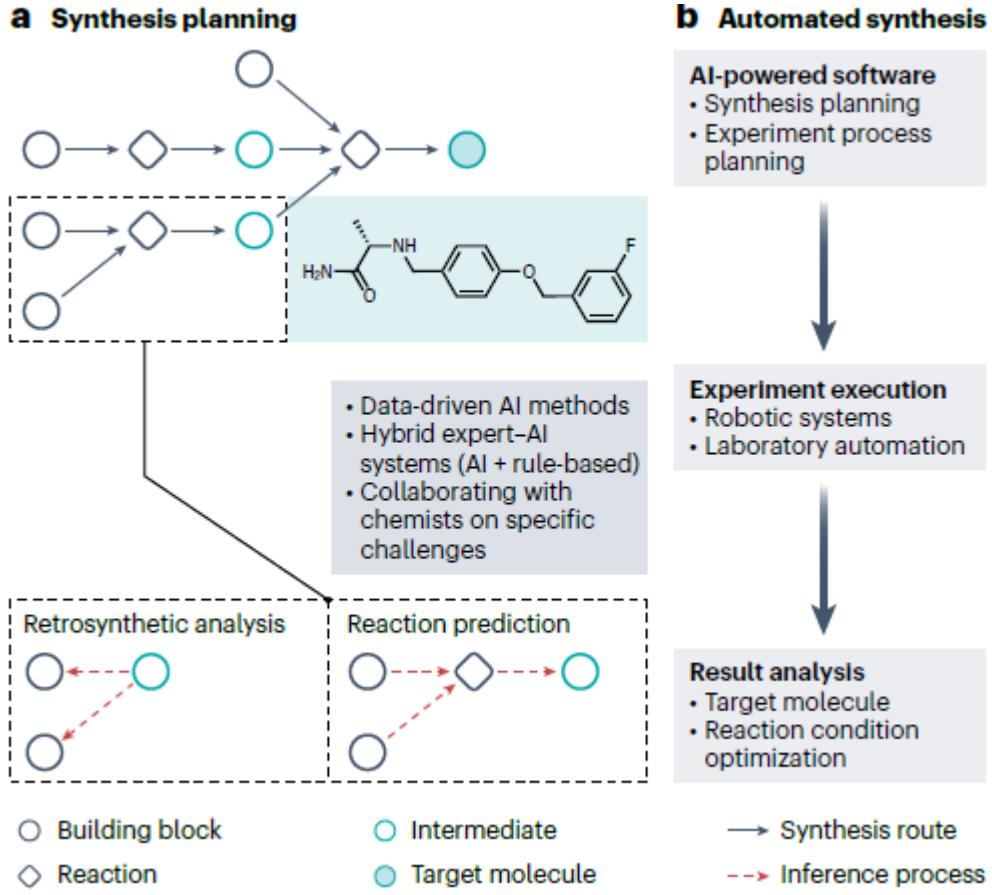
Kim and Khang, *Intern J of Nanomedicine* 2022

nature medicine

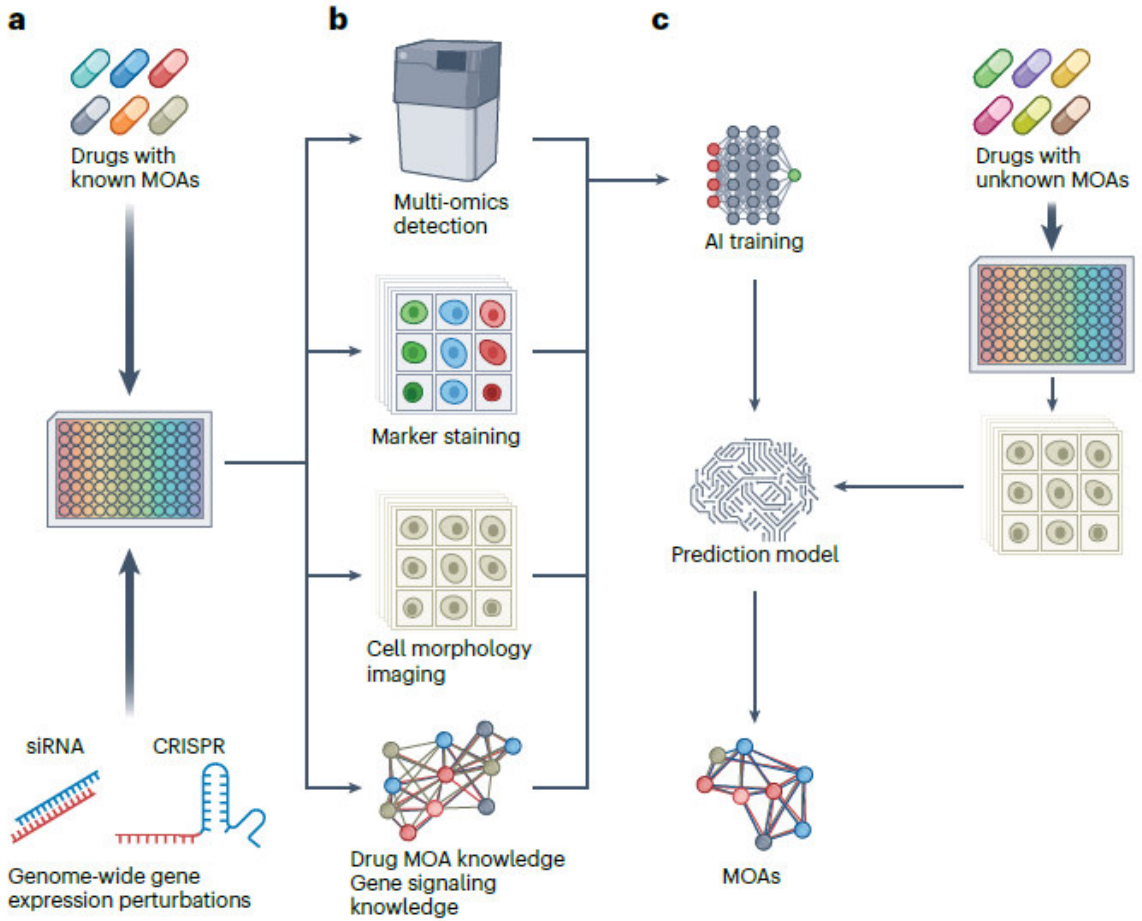
Review article https://doi.org/10.1038/s41591-024-03434-4

Artificial intelligence in drug development

AI-driven synthesis planning and automation in drug discovery



AI-driven MoA prediction using high-content screening and multiomics data



Zhang K et al., *Nature Medicine* Jan. 2025

AI in drug development and clinical trials

	Opportunities	Challenges
Target identification, drug development ✓ Target discovery, molecular design, lead optimization	Early days! New models, architectures	Experimental validation Inadequate models
❑ Response prediction, resistance mechanisms	Accelerate discovery 'Companion therapeutics'	Longitudinal biospecimens (tissue + liquid biopsies)
Patient stratification, diagnostics ✓ Digital pathology, radiology	Optimize & accelerate patient stratification Improve trial design	Biospecimens Data collection, sharing
❑ Multi-modal data integration	Leverage real-world data New models	Data harmonization Model bias