Microenvironment and metastatic cascade
Clinical Perspectives

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IEO
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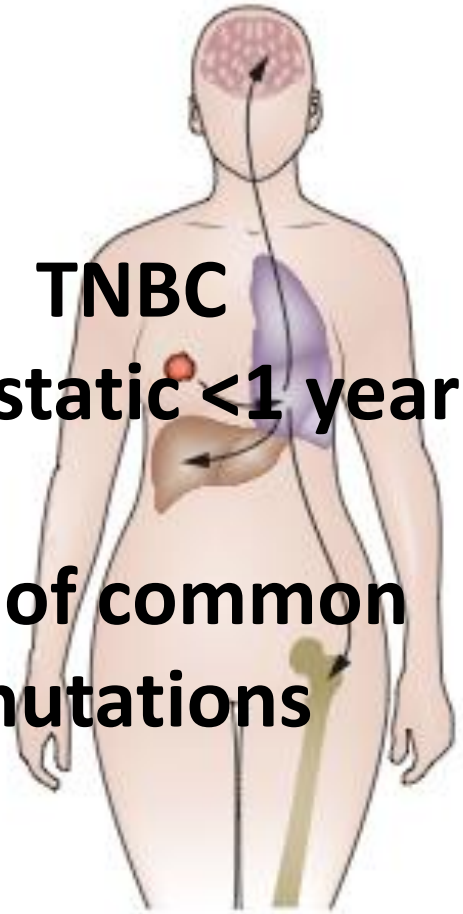
BIG against breast cancer

Tumor evolution in the metastatic setting

Christine Desmedt



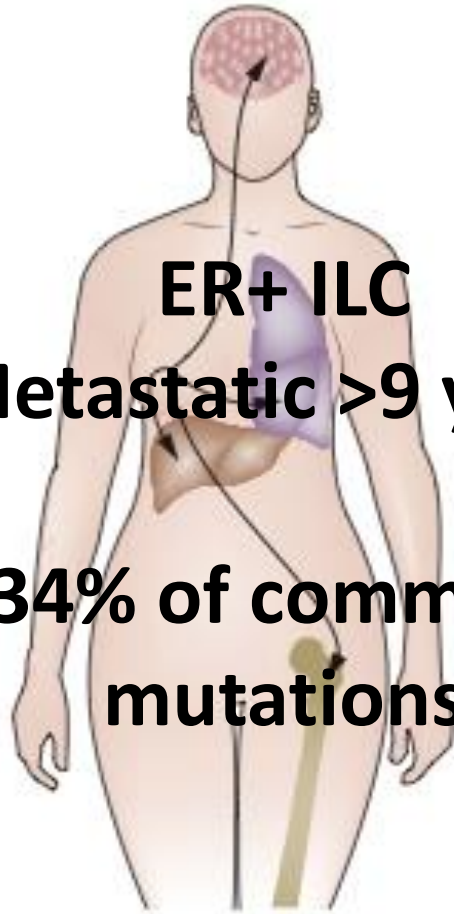
TNBC

Metastatic <1 year

96% of common mutations

Metastatic cascade

AND/OR?



ER+ ILC

Metastatic >9 years

34% of common mutations

Parallel progression

Naxerova & Jain, Nat Rev2015

Tumor evolution in the metastatic setting

- Autopsies → reconstruction of breast cancer progression.
- Different progression trajectories are possible in breast cancer (parallel and in cascade).
- Metastases can differ from their primary tumor, especially if the patients developed their metastases many years after initial diagnosis.

Tumor Evolution under Selective Therapeutic Pressure

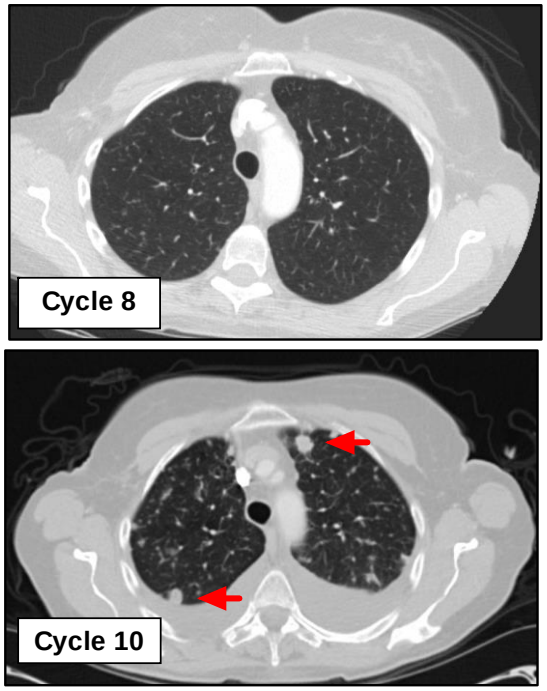
Tumor Evolution under Selective Pressure PI3Kα Inhibitor BYL719

1. **WGS of new lesion and primary**

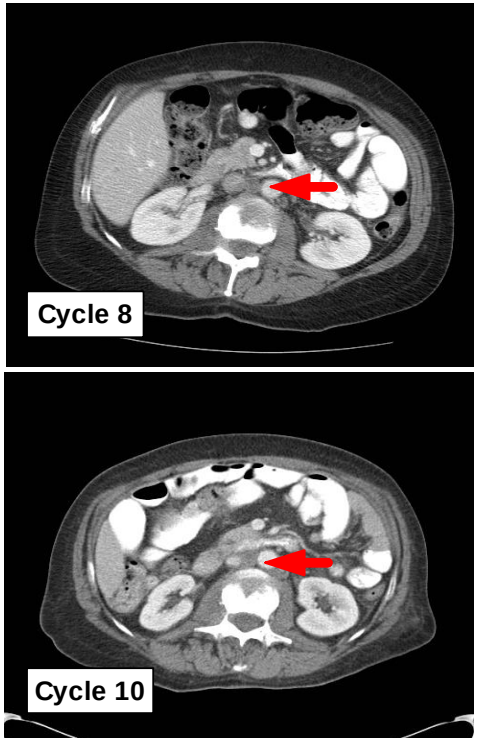
2. **WES of new, responding lesions and primary**

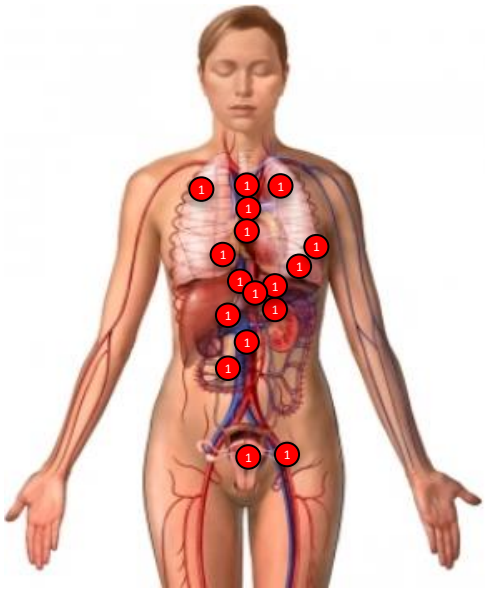
3. **Targeted exome sequencing of all metastases and primary**

PROGRESSION



RESPONDING



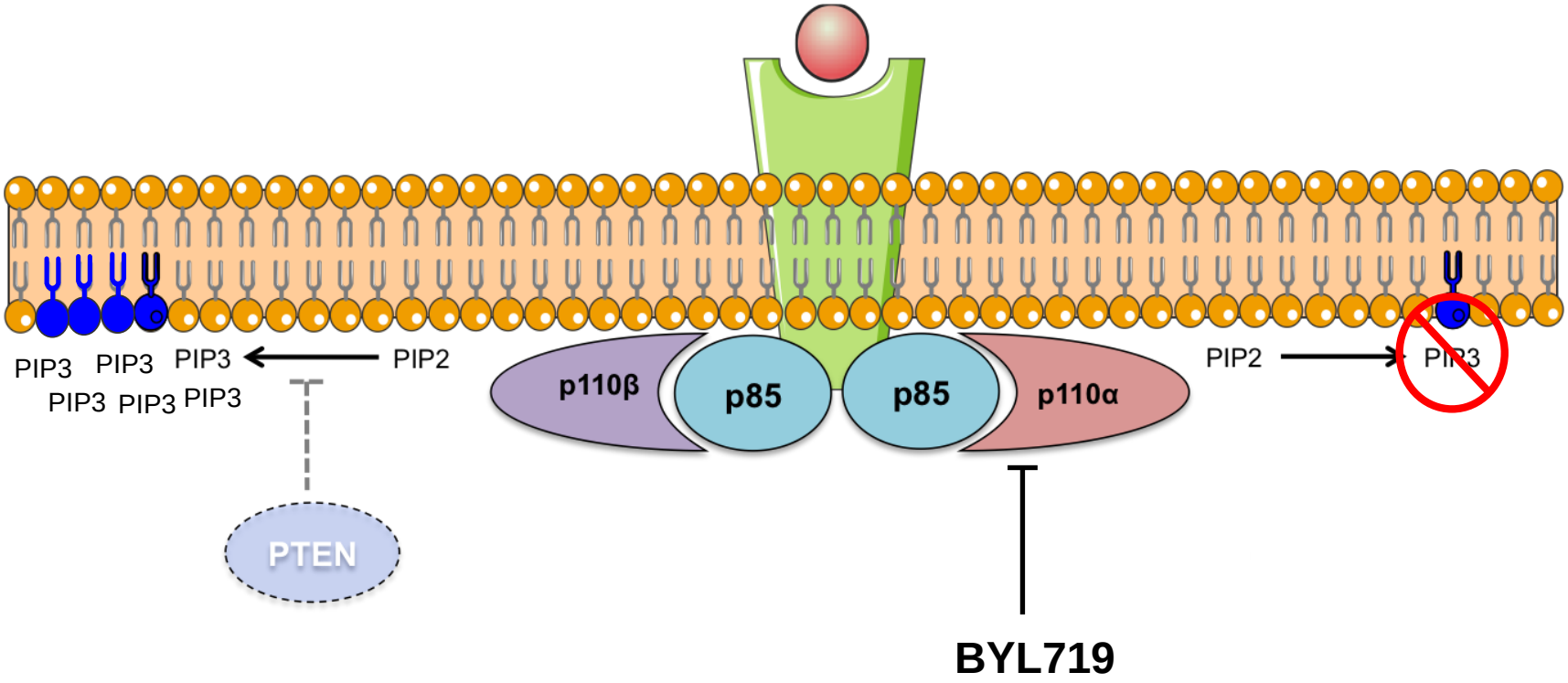


Juric, Castel et al. Nature 2015

Targeted exome sequencing reveals multiple PTEN alterations in all resistant lesions

Juric, Castel et al. Nature 2015

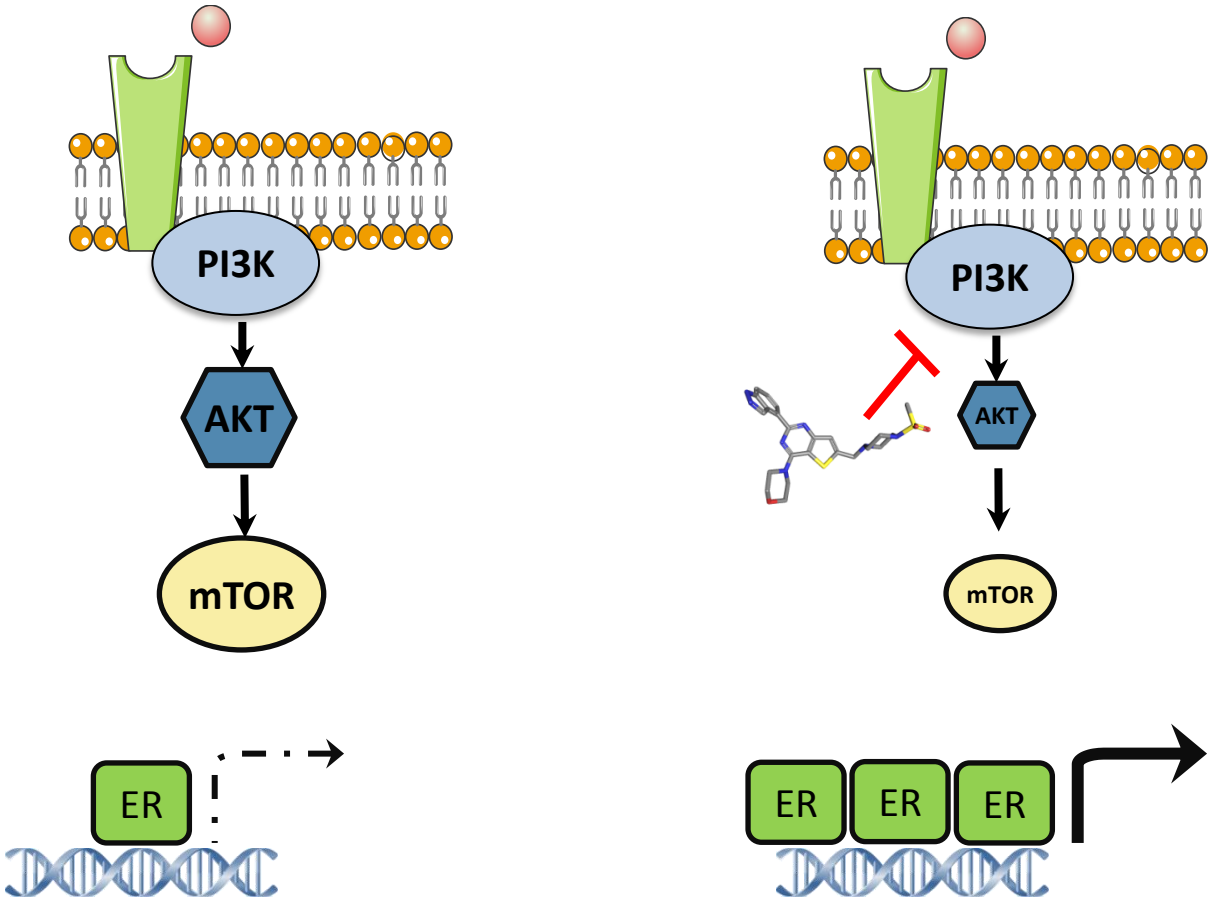
PTEN loss tumors activate AKT pathway through p110β



Jia *et al*. 2008, Nature

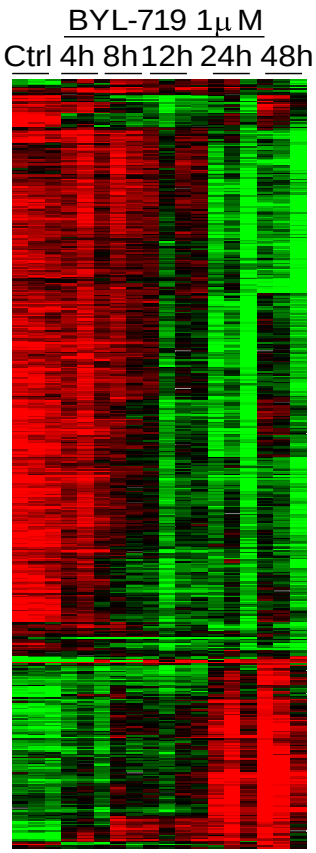
Wee *et al*. 2008, PNAS

PI3K inhibition augments estrogen receptor function and dependence in hormone receptor-positive breast cancer

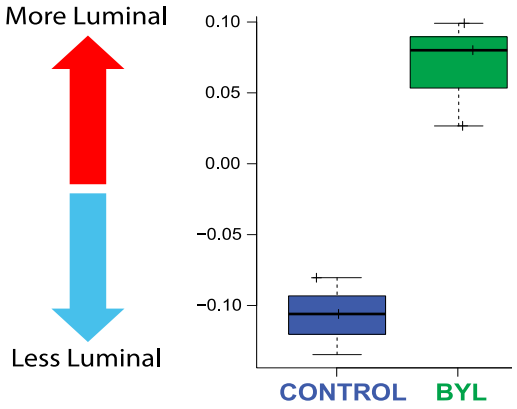


PI3Kα inhibition induces a transcriptome switch towards a more luminal (ER-driven) phenotype

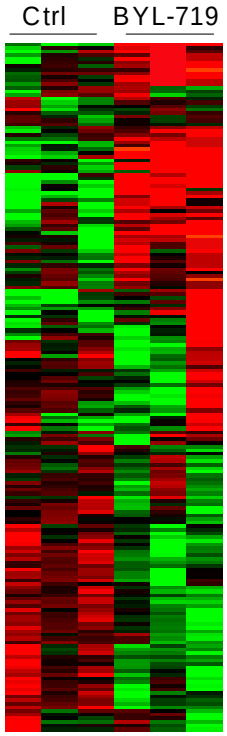
MCF7

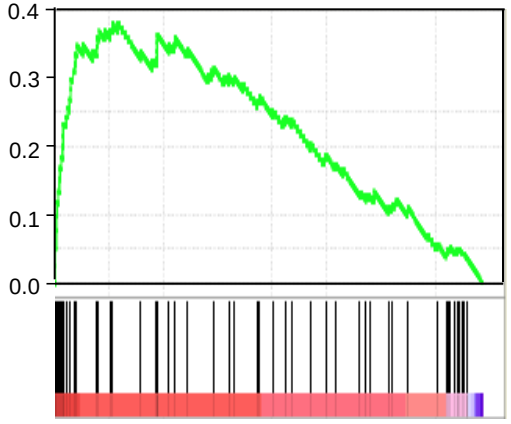


Enrichment Luminal signature



Patient Derived Xenograft





Enrichment after BYL-719

Bosch et al. *Sci Transl Med*. 2015

Clinical implications of tumor evolution

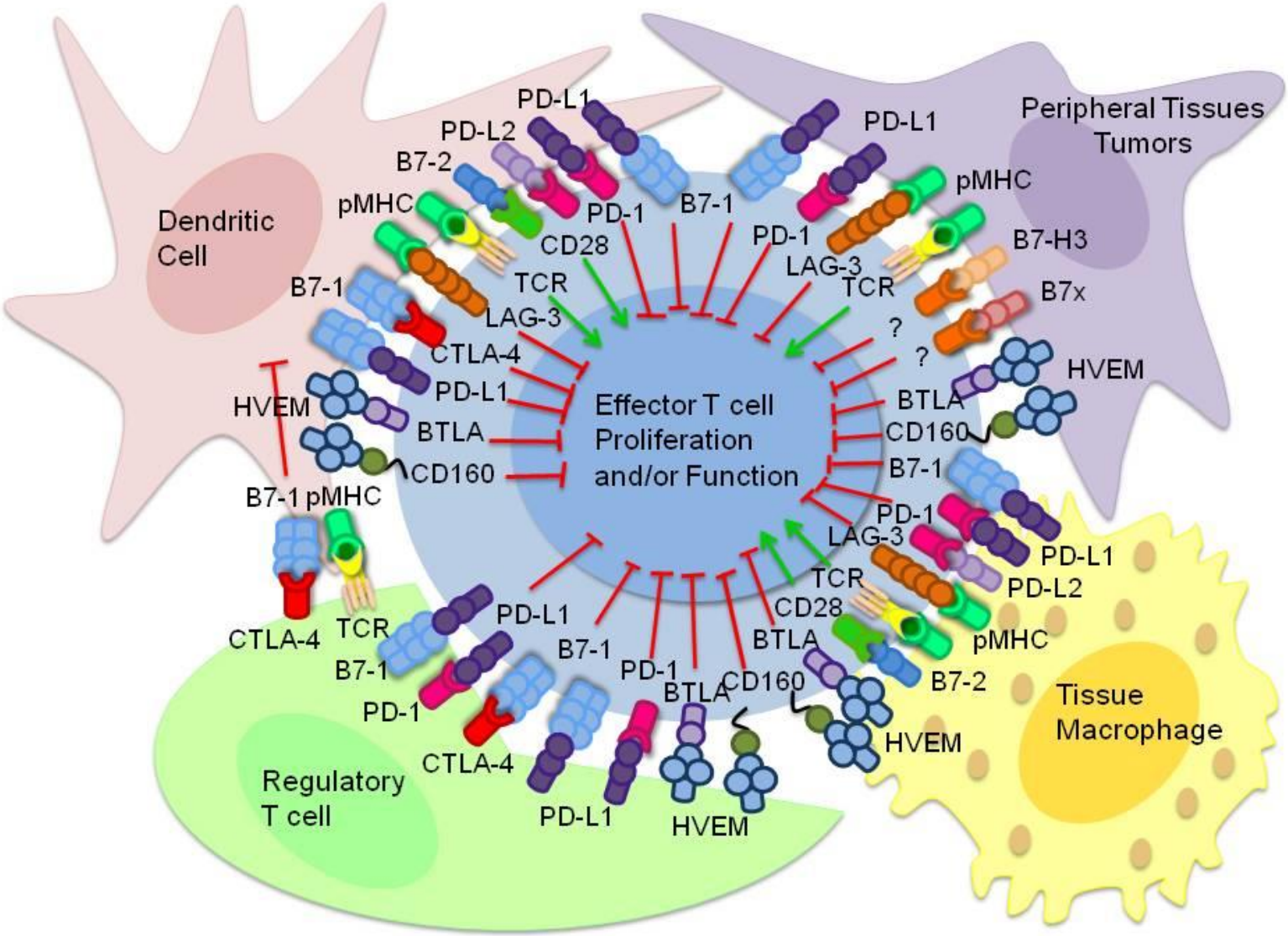
- PI3K mutations are frequent
- Selective PI3Kα inhibitors active in phase I in tumors with PI3Kα mutations
- Adaptive activation of ER occurs upon PI3k pathway inhibition
- SERDs and PI3Kα inhibitors in combination are very active. Registration trials under way
- Tumor evolution under selective pressure to be addressed

The impact of tumor genetics on host immune response

Matthew Hellmann

- Mutation burden, specific neoantigens, and patterns of neoepitopes may be a prediction tool
- Exome data can be used to identify neoantigen-specific T cell responses
- Neoantigen-specific T cells may mediate response to PD-1 blockade

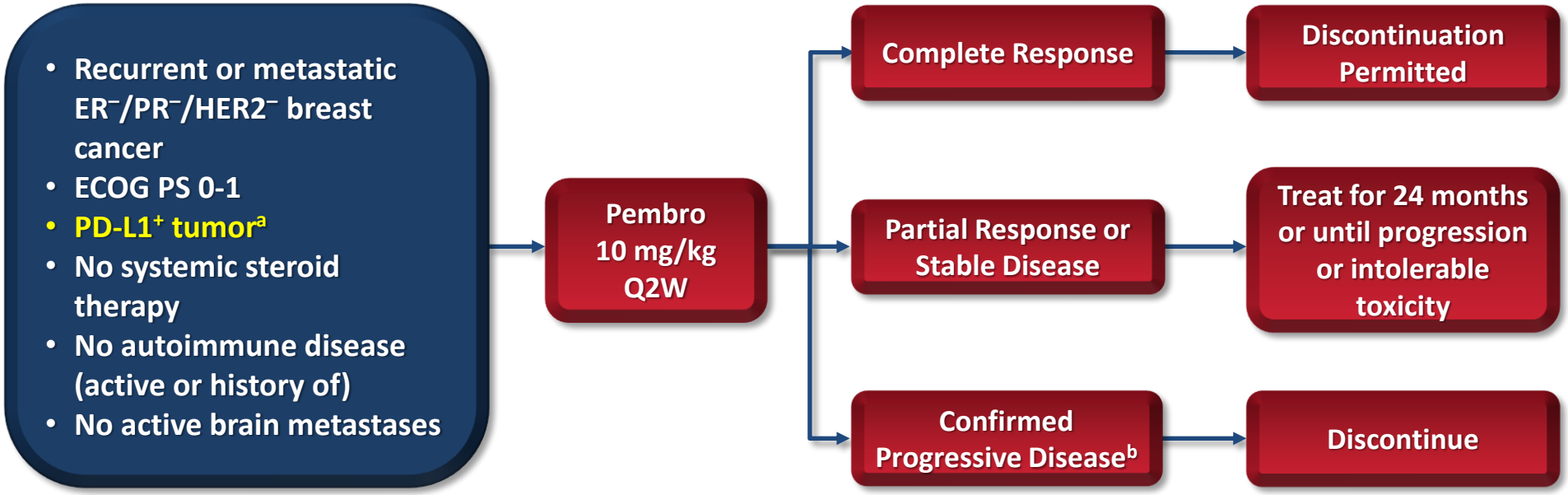
Predicting response



PD-L1 analysis: differences in evaluation and interpretation

Agent	Assay	Analysis	Definition of positivity	PD-L1 expression
Nivolumab (anti-PD-1) [1–4]	Dako automated IHC assay (28-8 rabbit Ab) Analytically validated	• Archival FFPE	• 1% and 5% cut-off among >100 evaluable tumour cells	• 56%: 1% cut-off • 49%: 5% cut-off
Pembrolizumab (anti-PD-1)[5,6]	Dako automated IHC assay (22C3 mouse Ab)	• Archival FFPE	• Tumour dependent: - Melanoma > 1% - NSCLC PD-L1 (+): Strong (≥50%) and weak staining (1–49%) PD-L1 (–): no staining	• ~25%: ≥50% staining • ~45–70%: ≥1% staining
MPDL3280A (anti-PD-L1)[7,8]	Ventana automated clinical research IHC assay	• Archival FFPE	• PD-L1 (+): IHC 3 (≥10%), IHC 2,3 (≥5%), IHC 1,2,3 (≥1%) • PD-L1 (–): IHC 1, 0 or unknown	• 11%: IHC 3 • 75%: IHC 1, 0
MEDI-4736 (anti-PD-L1)[9]	First-generation or Ventana IHC Automated Assay (in dev.)	• Archival FFPE	• Not reported	• Not reported

The immune checkpoint inhibitors



- **PD-L1 positivity:** 58% of all patients screened had PD-L1-positive tumors
- **Treatment:** 10 mg/kg IV Q2W
- **Response assessment:** Performed every 8 weeks per RECIST v1.1

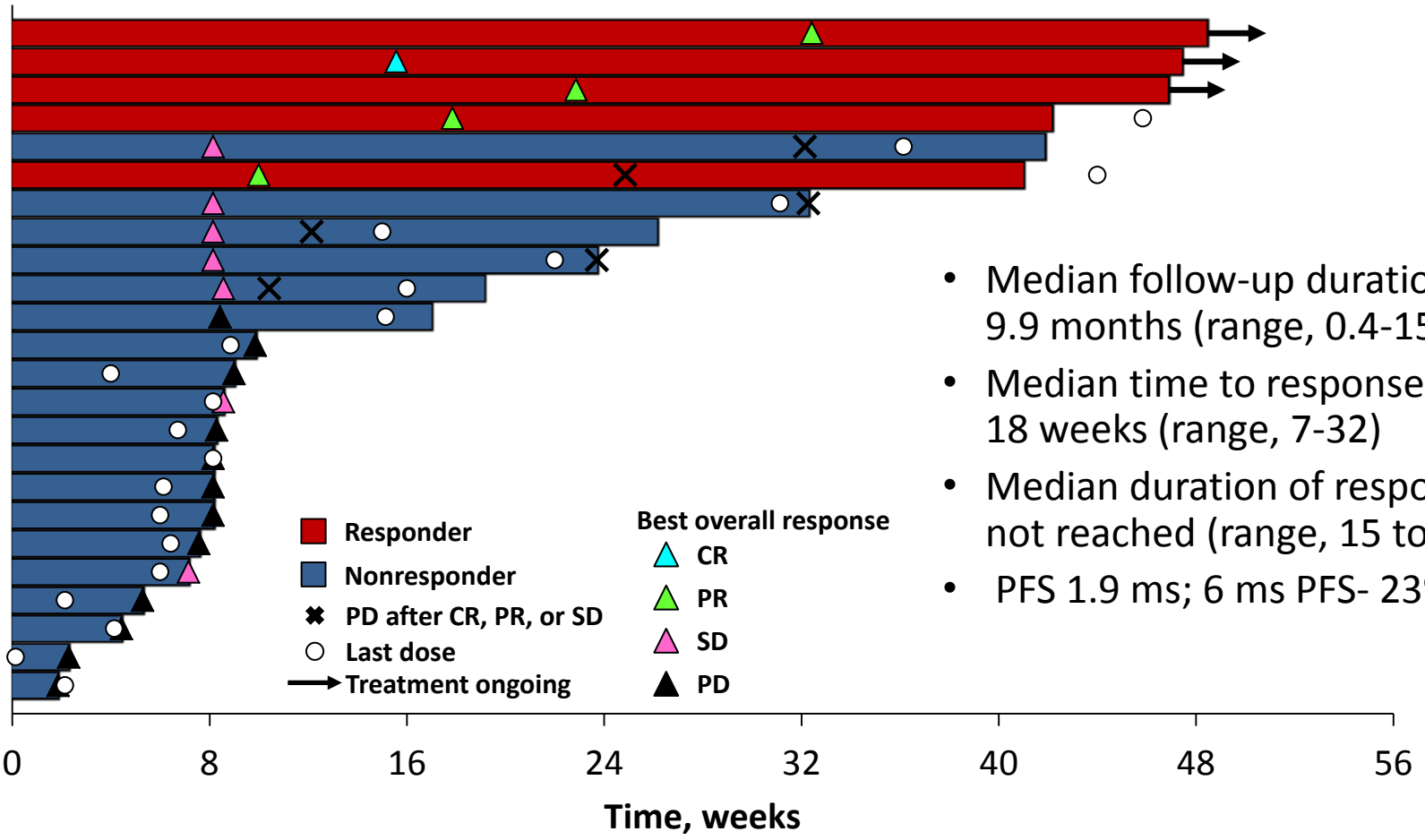
[a]PD-L1 expression was assessed in archival tumor samples using a prototype IHC assay and the 22C3 antibody. Only patients with PD-L1 staining in the stroma or in ≥1% of tumor cells were eligible for enrollment.
[b]If clinically stable, patients are permitted to remain on pembrolizumab until progressive disease is confirmed on a second scan performed ≥4 weeks later. If progressive disease is confirmed, pembrolizumab is discontinued. An exception may be granted for patients with clinical stability or improvement after consultation with the sponsor.

Pembrolizumab in TN breast cancer

	Patients Evaluable for Response[a] n = 27
Overall response rate	5 (**18.5**%)
Best overall response	
Complete response[b]	1 (3.7%)
Partial response[b]	4 (14.8%)
Stable disease	7 (25.9%)
Progressive disease	12 (44.4%)
No assessment[c]	3 (11.1%)

Nanda, SABCS 2014

Pembrolizumab in TN breast cancer



- Median follow-up duration: 9.9 months (range, 0.4-15.1)
- Median time to response: 18 weeks (range, 7-32)
- Median duration of response[a]: not reached (range, 15 to 40+ weeks)
- PFS 1.9 ms; 6 ms PFS- 23%

[a]Kaplan-Meier estimate.
Analysis cut-off date: November 10, 2014.

Nanda, SABCS 2014

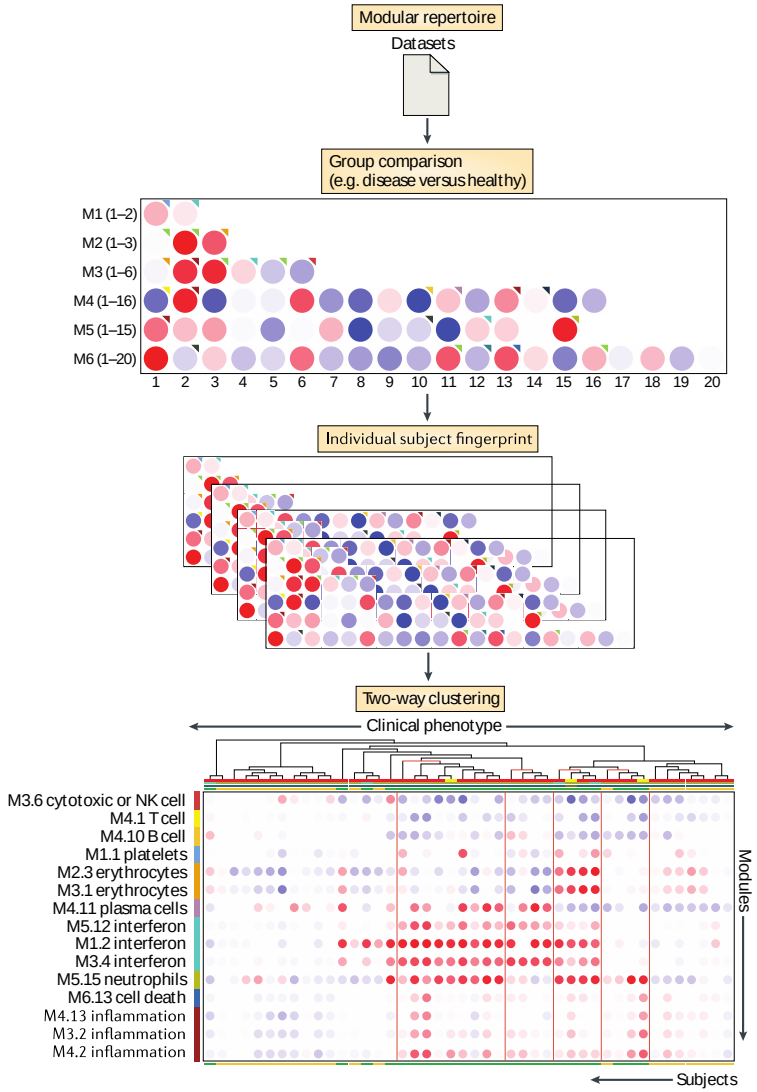
PD-L1 as a biomarker

Drug/ Sponsor	Nivolumab BMS			Pembrolizumab MSD (Merck)			MPDL3280A Genentech			MEDI4736 MedImmune
Assay	28-8			22C3			Dako			SP263
Cells scored	Tumor cell membrane			Tumor cell (and stroma)			Infiltrating immune cells			Tumor cells
Tissue	Archival			Recent			Arch./Recent			Arch./Recent
Cut-point	**5%**	**1%**	**5%**	**1%**	**1%**	**50%**	**1%**	**5%**	**10%**	**10%**
ORR in PD-L1 +	50% N=10	13% N=38	15% N=33	26-47% N=45	19-23% N=177	37% N=41	31% N=26	46% N=13	83% N=6	39% N=13
ORR in PD-L1 -	0% N=7	17% N=30	14% N=35	???	9-13% N=40	11% N=88	20% N=20	18% N=33	18% N=40	5% N=19

Clinical Considerations

- PD-L1 expression is dynamic
- PD-L1 is heterogeneous within tissue
- PD-L1 “threshold” is to be defined (tumour material, mAB, technique, sampling, criteria)
- Importance of co-localization with TILs

Democratizing systems immunology with modular transcriptional repertoire analyses



Mapping perturbations of the modular repertoire across individual samples.

Mapping perturbations of the modular repertoire for a group of subjects does not account for the heterogeneity observed at the individual level.

Modular fingerprints can be derived for individual subjects using a reference set of samples.

Damien Chaussabel and Nicole Baldwin, Nature Immunology, 2014

Modulate the Immune System in Cancer: Checkpoint inhibitors

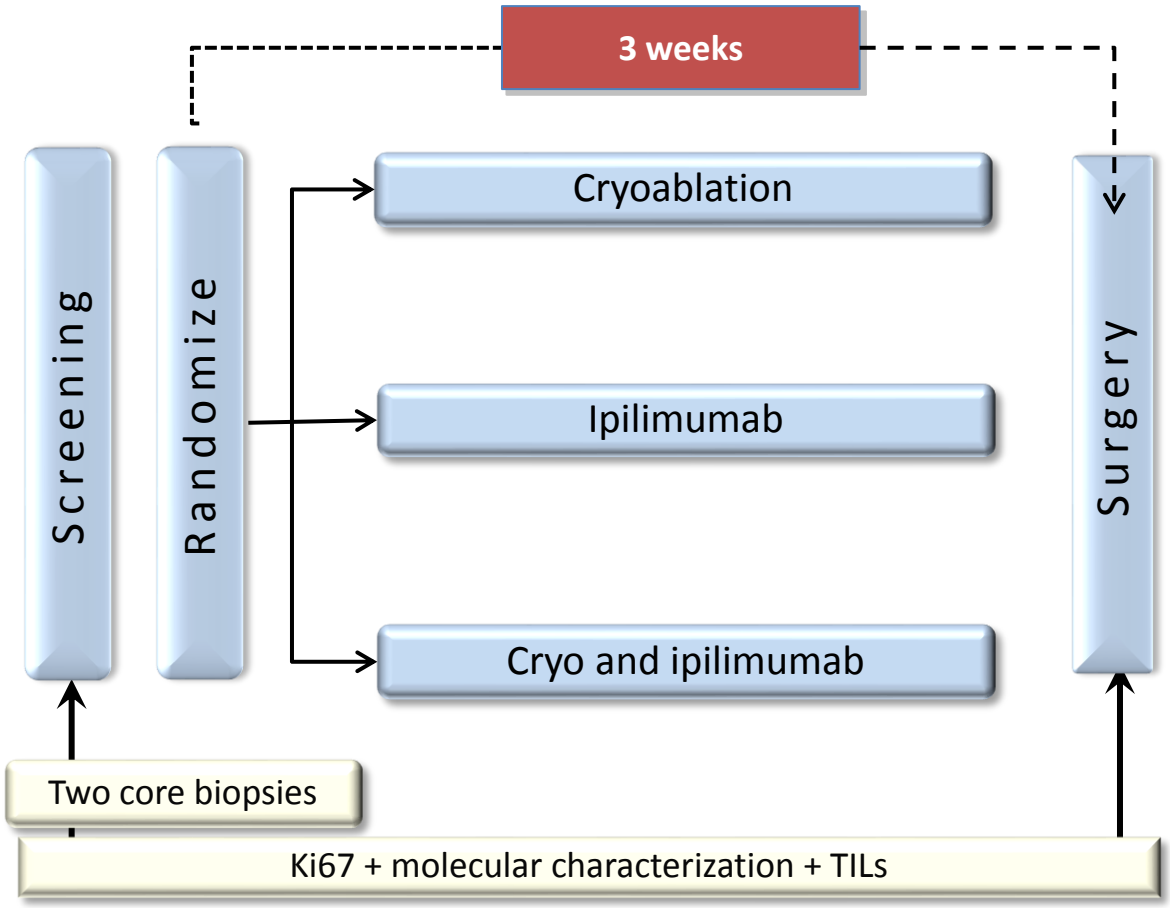
Karen Willard-Gallo

Tertiary Lymphoid Structures (TLS) produce memory T and/or B cells that function to reject allografts

Vaccinated patients form TLS in the stroma adjacent to their lesion

TIL_{hi} cancer patients with TLS have a better prognosis (due to quality as well as quantity of memory T and B cells?)

Can TLS be induced in the tumor site? "Abscopal effect" in breast cancer



- Resectable tumor
- Diameter ≥1.5 cm by ultrasound
- Grade II-III

Primary Endpoint
- Proliferation: Ki67
- TILs following treatment

Secondary Endpoints
Systemic Inflammatory response

Diab A. et al. J Clin Oncol 32:5s, 2014 (suppl; abstr 1098)

Activating antitumor immunity with cryoablation and ipilimumab in EBC

- Tissue biopsies and cryoablation were performed 7-10 days prior to surgery. Ipilimumab was administered 8-15 d prior to mastectomy
- Pre-operative cryo-alone, ipi-alone and the combination were well tolerated and the primary safety endpoint was achieved.

Diab A. et al. J Clin Oncol 32:5s, 2014 (suppl; abstr 1098)

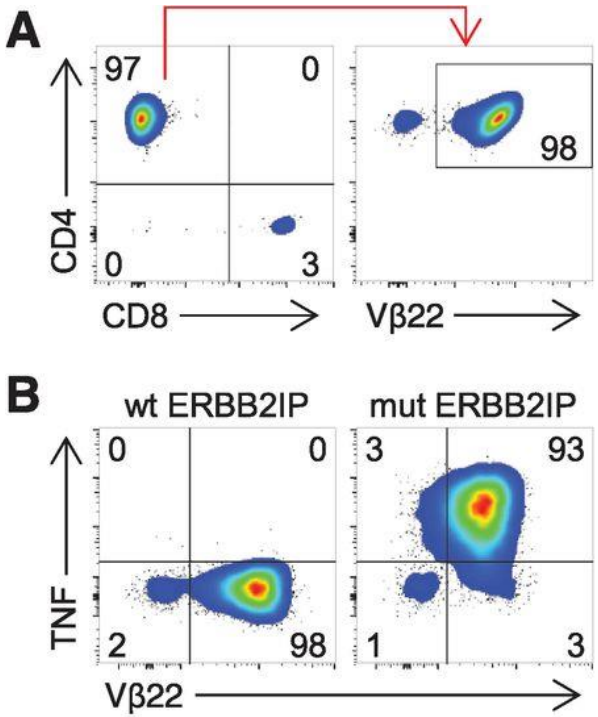
Activating antitumor immunity with cryoablation and ipilimumab in EBC

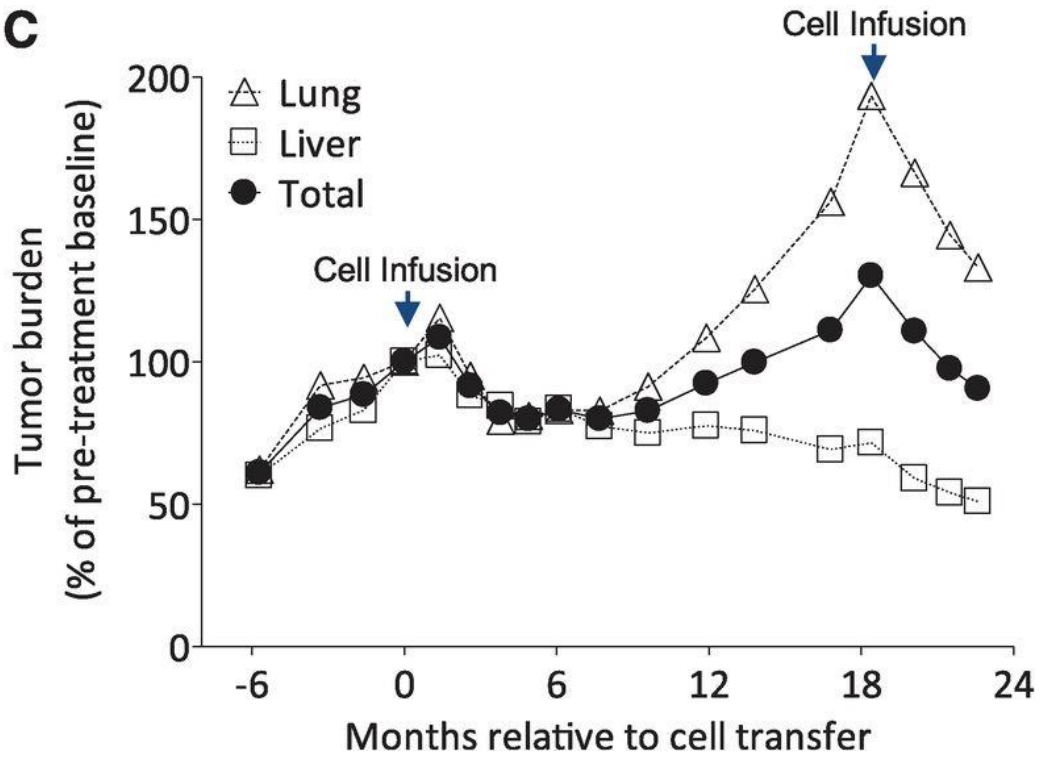
- Tumor necrosis/infarction was observed in 9/12 pts who underwent cryoablation.

- Analysis of TILs in the TM specimens suggested a higher ratio of CD8+Ki67+ T-cells to CD4+CD25+FOXP3+ (T-regulatory) cells in group C (cryo+ipi) when compared with cryo alone and ipilimumab alone.

Diab A.et al. J Clin Oncol 32:5s, 2014 (suppl; abstr 1098)

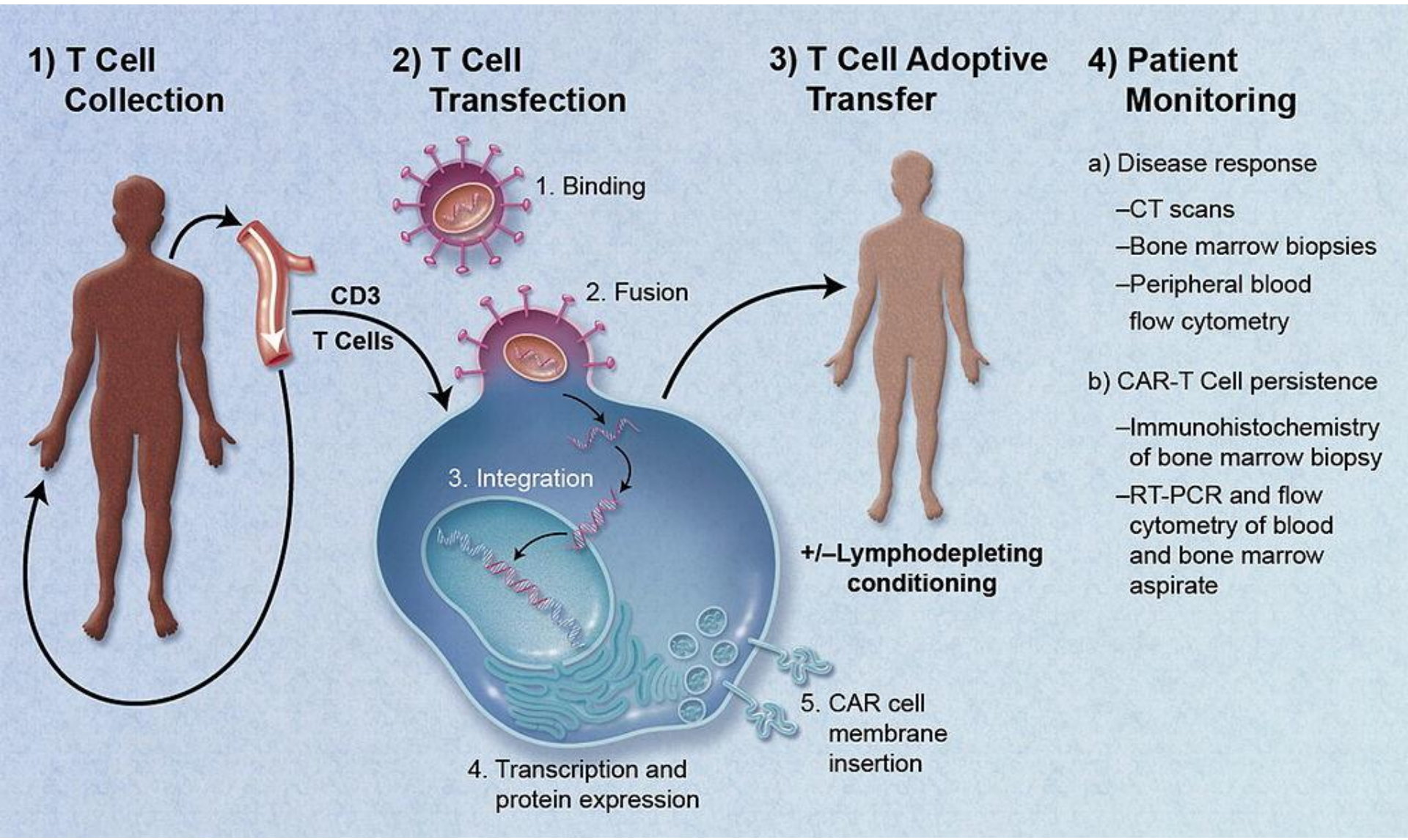
T-cell therapy against cancer mutations





Tran E et al Science. 2014, 9;344(6184):641-5

Chimeric T cell receptors



Morgan R et al Mol Ther. Apr 2010; 18(4): 843–851

Predicting immune-response in breast cancer

- The more "immunogenic" → higher likelihood to respond

- How to define "immunogenic"?:
  - TILs (which cut-off?)
  - TLS present or absent?
  - Immune determinants (neo-antigens)?
  - PD1/PD-L1 expression (unclear)?

Clinical Considerations

- Somatic neo-epitopes are shared by patients with a prolonged benefit from ICPI and are absent in those without a prolonged benefit
- We need for an expanded definition of the previous categories of “driver” and “passenger” mutations.
- Neoantigens may represent “immune determinants.”
- Mapping perturbations of the modular immunotrascriptomic repertoire to address subjects heterogeneity

Thank you

Path and Stat
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Maria Angela Massaro
Concetta Riviello

AIRC

Fondazione Umberto Veronesi
PER IL PROGRESSO DELLE SCIENZE

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