ASSOCIAZIONI o SEQUENZE:

Il punto di vista dell'immunologo

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Modulo dichiarazione conflitto di interessi

Tutti i rapporti finanziari intercorsi negli ultimi due anni devono essere dichiarati.

☒ Non ho rapporti (finanziari o di altro tipo) con le Aziende del farmaco

☐ **Ho / ho avuto rapporti (finanziari o di altro tipo) con le Aziende del farmaco**

Relationship	**Company/Organization**
Research grant	IPSEN
Research Grant	Novartis
Research Grant	Incyte

State of art in immunoncology: the perspective of the immunologist

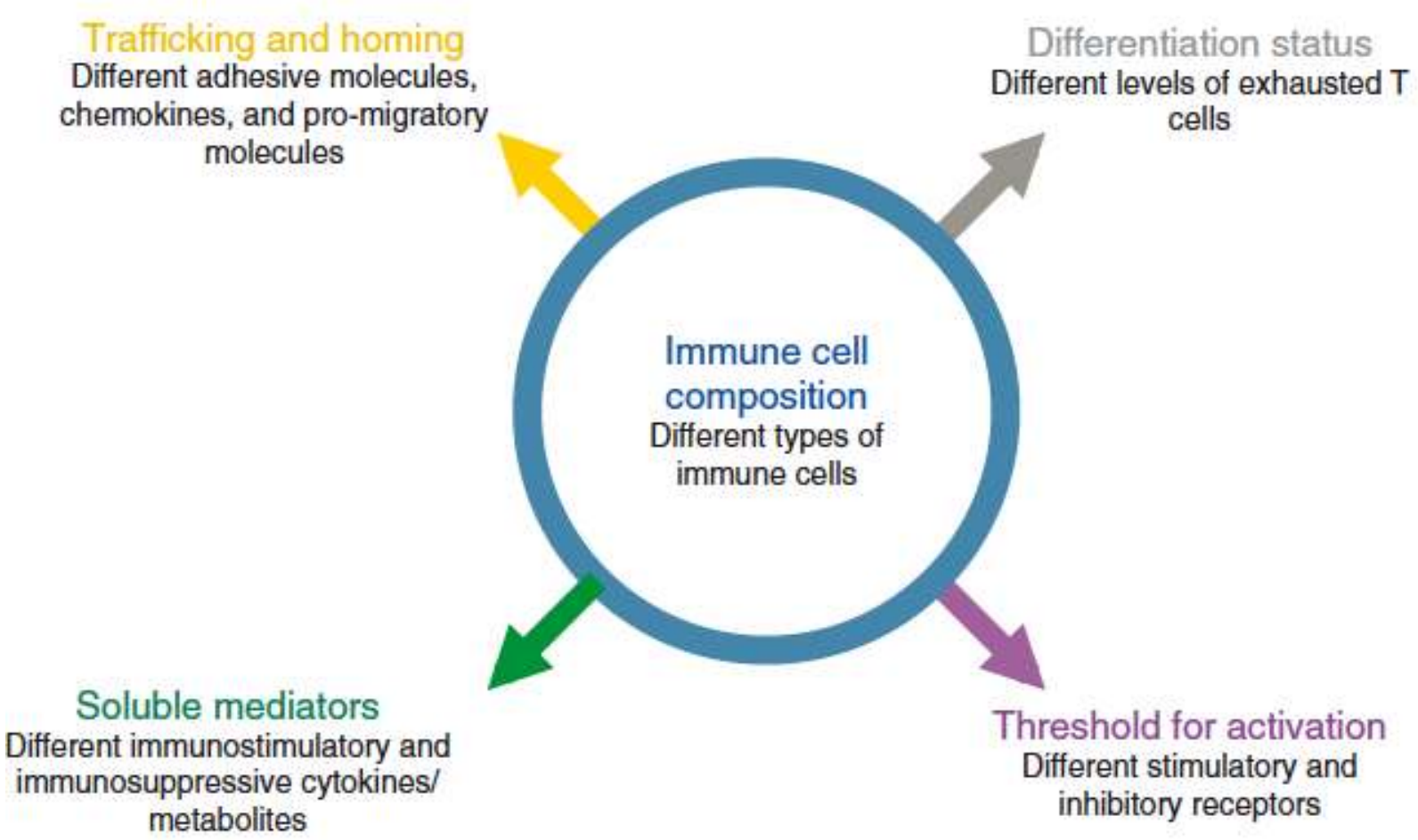
- The established GOOD news: T cells can kill and control tumor progression
  - Successfull clinical trials in all cancers
  - Adoptive cell therapy
  - CAR-T therapies
- Responder pts prolongued survival, partial responders/stable disease, Non responder (hyperprogressor): we are not giving the wright treatment to each pt….
- Need to acquire lessons from these pts

Immunity of cancer pts changes during tumor progression and during treatment

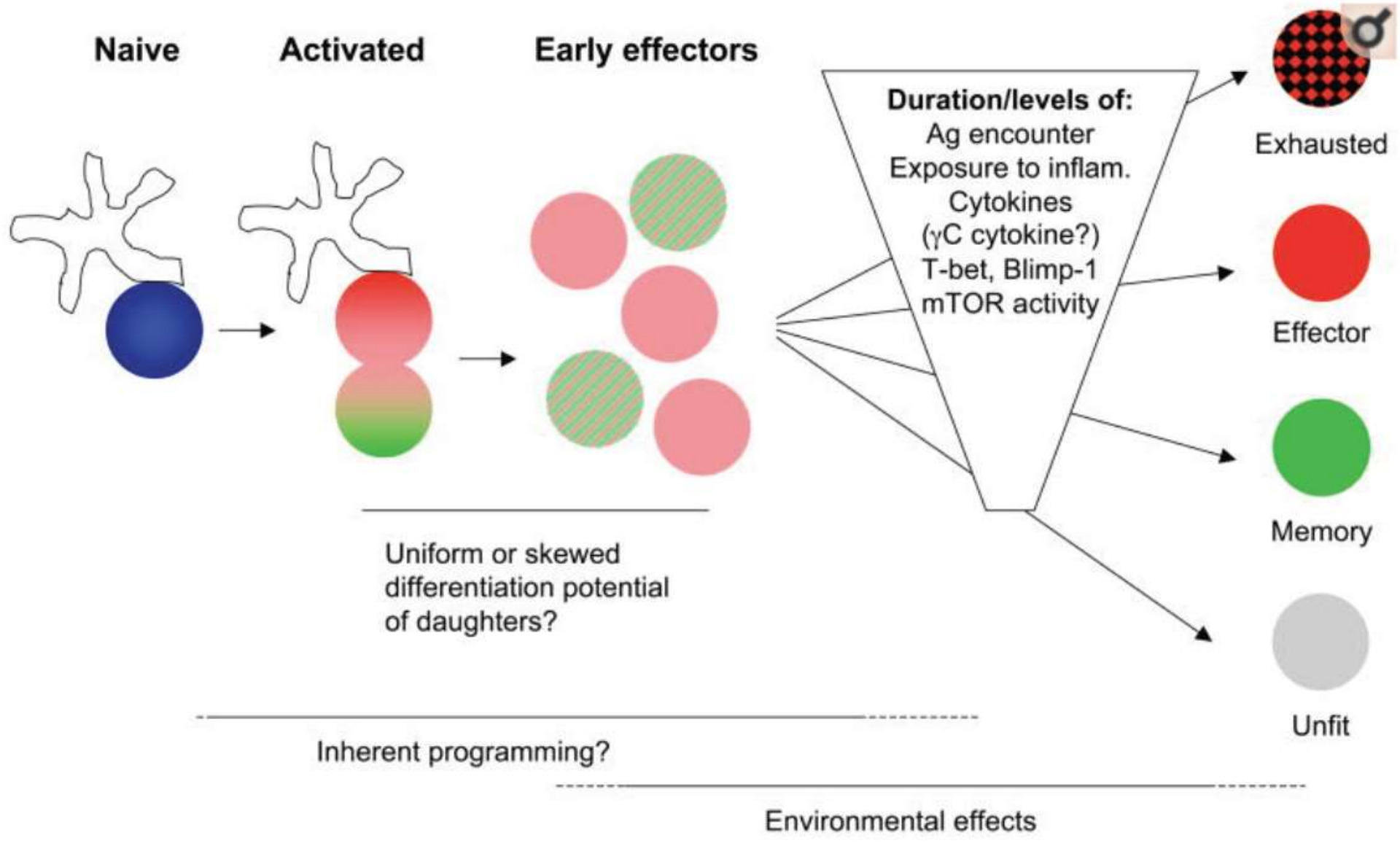
- Pts display different immunocompetence (fitness)
- “Best predictive/prognostic factor is the treatment” cit Mirabelle
- Dynamic and patient related biomarkers are the only indicators of (efficacy/non efficacy) activity of the treatment
- Need to test different combination and sequences monitoring immune effects to define novel protocols of treatment

FIRST VARIABLE:
TUMOR HISTOTYPE, ORGAN IMMUNITY and TME

The immune system is organ specific

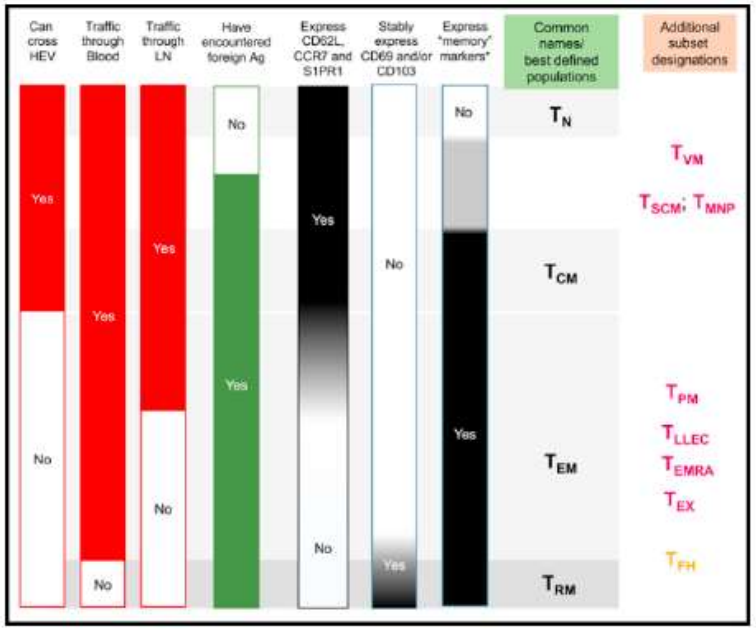


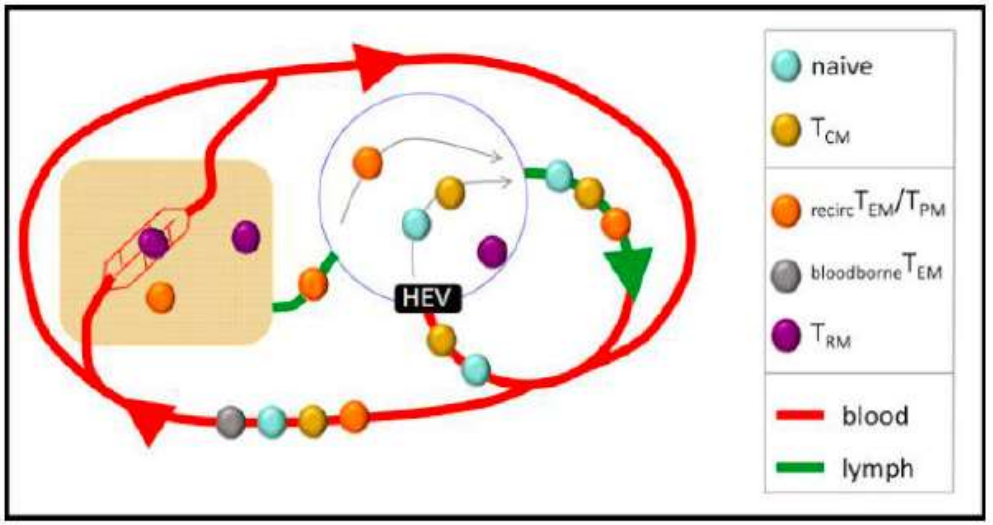
Pao, Cancer Discovery 2019


Naive
Activated
Early effectors
Duration/levels of:
Ag encounter
Exposure to inflam.
Cytokines
(γC cytokine?)
T-bet, Blimp-1
mTOR activity
Exhausted
Effector
Memory
Unfit
Uniform or skewed
differentiation potential
of daughters?
Inherent programming?
Environmental effects

Different T cells have the capacity to recirculate and traffick to the tumor

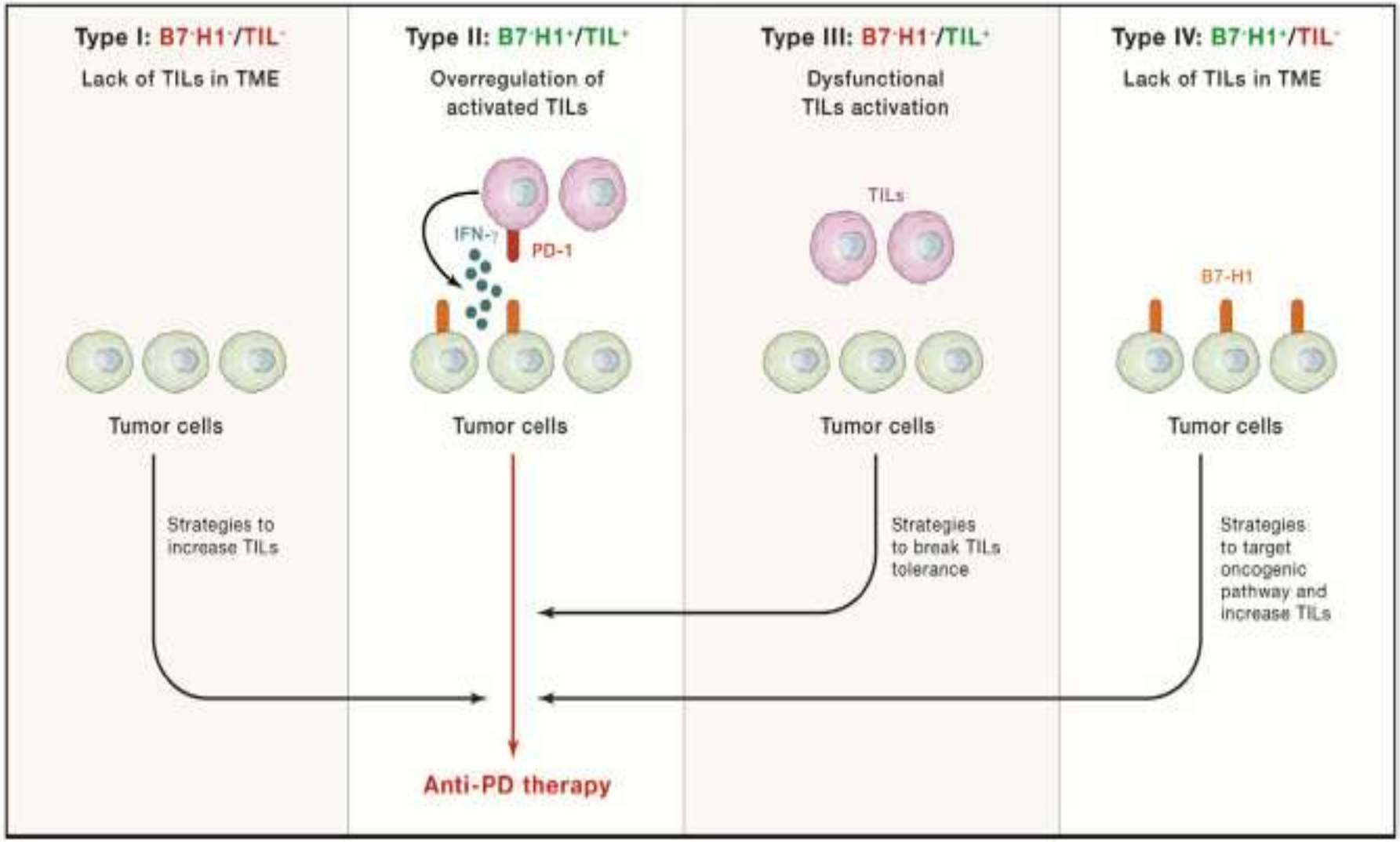
Step 4: Trafficking of T cells to the tumor





Jameson, Immunity 2019

Tumor immunity in the TME classification



Immunological ignorant tumor

Adaptive resistance in the tumor

TILs tolerance in the tumor No IFNγ

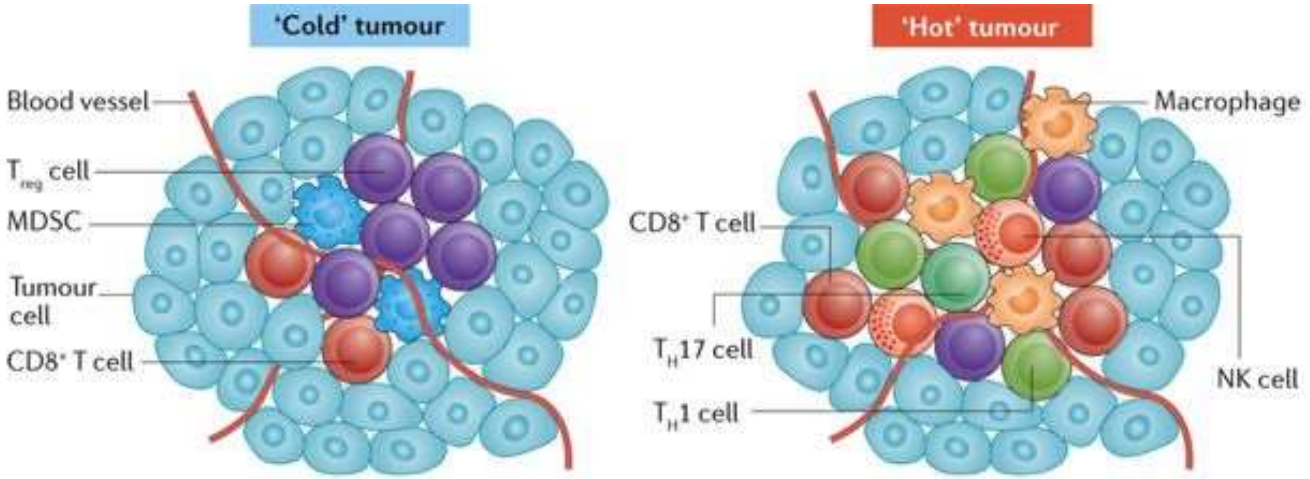
Intrinsic induction of PDL-1 (signalling)

Sanmamed M, Cell 2018

The first interaction of the tumor with the immune system dictates

possibility to respond to immunotherapeutic drugs

--IL MICROMABIENTE IDENTIFICA UN TUMORE FREDDO DA UN TUMORE CALDO



	'Cold' tumour	'Hot' tumour
Biological characteristics	• Epigenetic silencing • Active β-catenin signalling • Mesenchymal-like cells • Stem cell-like cells • Less-differentiated cells	• Epigenetic reprogramming • Suppressed β-catenin signalling • Epithelial cells • Highly differentiated cells • High PDL1 expression
Immunological characteristics	• Enriched in immunosuppressive cytokines • High numbers of T_{reg} cells and MDSCs • Few T_H1 cells, NK cells and CD8⁺ T cells • Few functional APCs	• Enriched in T_H1-type chemokines • High numbers of effector immune cells (T_H1 cells, NK cells and CD8⁺ T cells) • High numbers of functional APCs

Nature Reviews | Immunology

- ✓ **Chemotherapy/target therapy** aids in fast tumor regression and **reduction tumor burden**

- ✓ **ICI** prolong this effect inducing **a long lasting antitumor response.**

Guido Kroemer and Laurence Zitvogel: Chemo and target therapies need an intact immune system for efficacy

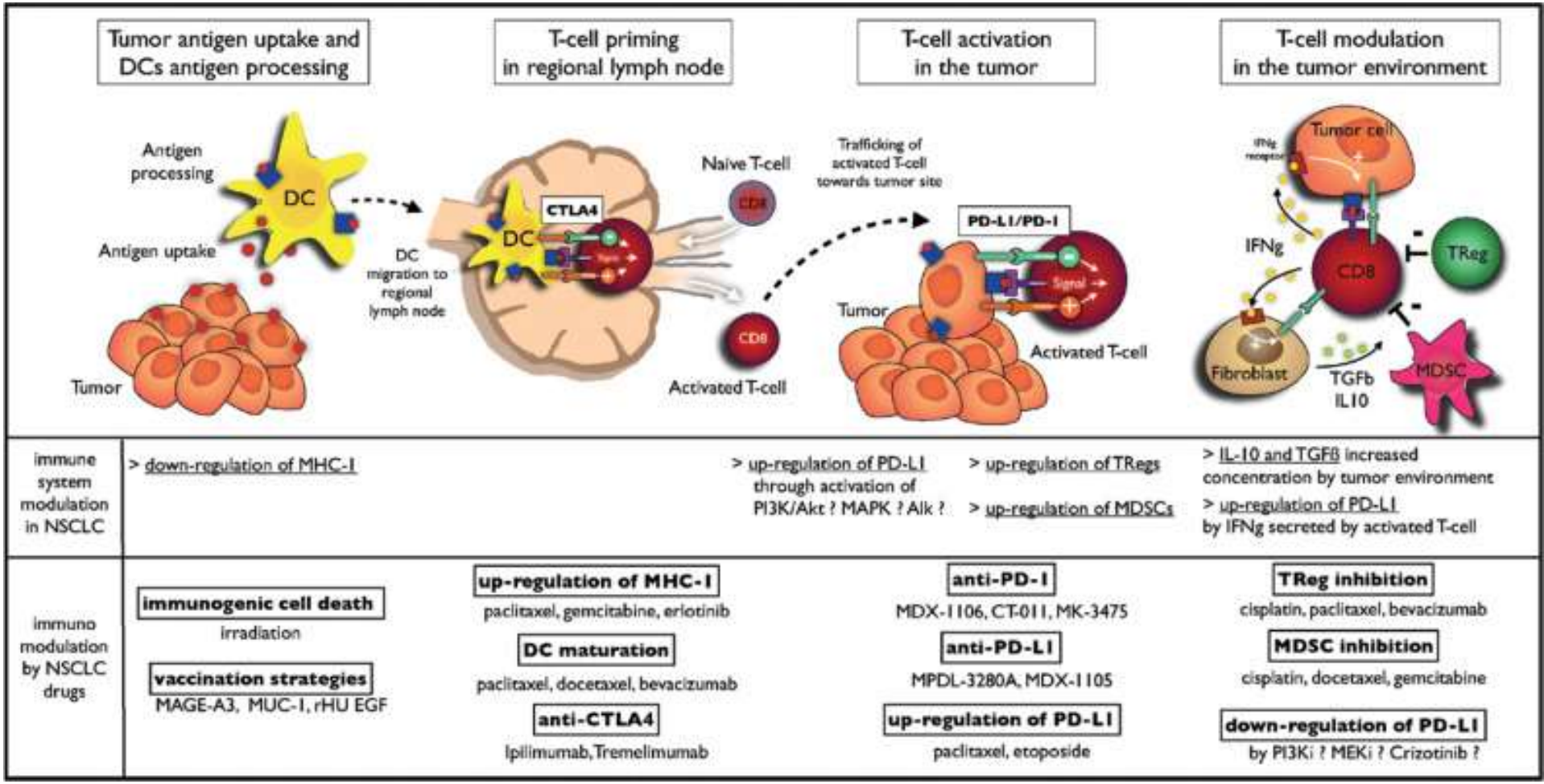
Chemotherapy can boost the immune system

Table 1
Immune modulating properties of chemotherapeutic agents commonly used for NSCLC treatment.

Type	Agent	Immune modulation mechanism	Reference
Platinum alkylating agents	Cisplatin	Increased T cell recognition, CTL-mediated attack (when combined with vinorelbine)	(De Biasi et al., 2014; Goss and Tsvetkova, 2012; Hato et al., 2014; Mathew et al., 2018; Wang et al., 2018a, 2018b; Wu and Waxman, 2018; Zitvogel et al., 2008)
	Carboplatin	Increased T cell recognition	(Bezu et al., 2015; De Biasi et al., 2014; Goss and Tsvetkova, 2012; Hato et al., 2014; Mathew et al., 2018; Roselli et al., 2013; Wang et al., 2018a, 2018b; Wu and Waxman, 2018; Zitvogel et al., 2008)
Spindle poisons (taxanes)	Paclitaxel	Enhanced expression of MHC class II, DC maturation, decreased Tregs, increased function of CD4+ and CD8+	(John et al., 2010; Zhang et al., 2008; Zitvogel et al., 2008)
	Docetaxel	Depletion of Tregs, activation of STAT3 signaling, reduced MDSCs	(Champiat et al., 2014; John et al., 2010; Kodumudi et al., 2010; Zhang et al., 2008; Zitvogel et al., 2008)
Antimetabolites	Pemetrexed	Increased T cell infiltrate and antigen presentation, activation of CD45RO + memory T cells and IFN-γ producing NK cells	(Mathew et al., 2018; Novosiadly et al., 2018; Tomasini et al., 2016)
	Gemcitabine	Enhanced expression of tumor-antigens, selective MDSC repression	(Galluzzi et al., 2017; Mathew et al., 2018; Novosiadly et al., 2018; Tomasini et al., 2016; Weir et al., 2011)
Anthracyclines	Doxorubicin	Increased presence of T cells and T cell chemokines, ICD-inducer	(Hopewell et al., 2013; Mi et al., 2008; Wang et al., 2018a, 2018b; Wu and Waxman, 2018; Zitvogel et al., 2008)

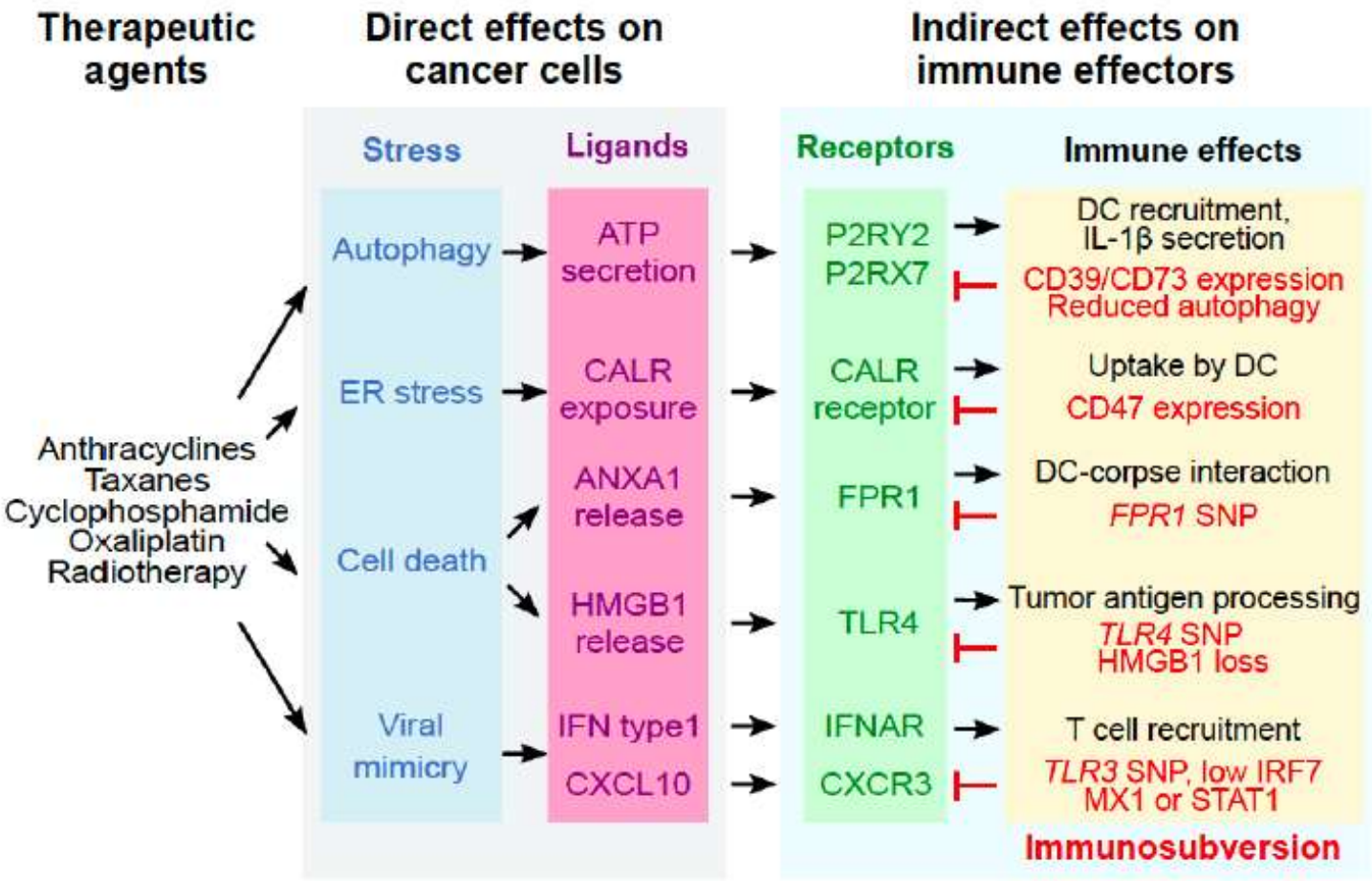
CTL = cytotoxic T lymphocytes; DC = dendritic cell; ICD = immunogenic cell death; MDSC = myeloid-derived suppressor cell; NK cell = natural killer cell; STAT = signal transducers and activators of transcription, Treg = regulatory T cell.

NSCLC tumor immunology and modulation by conventional therapies

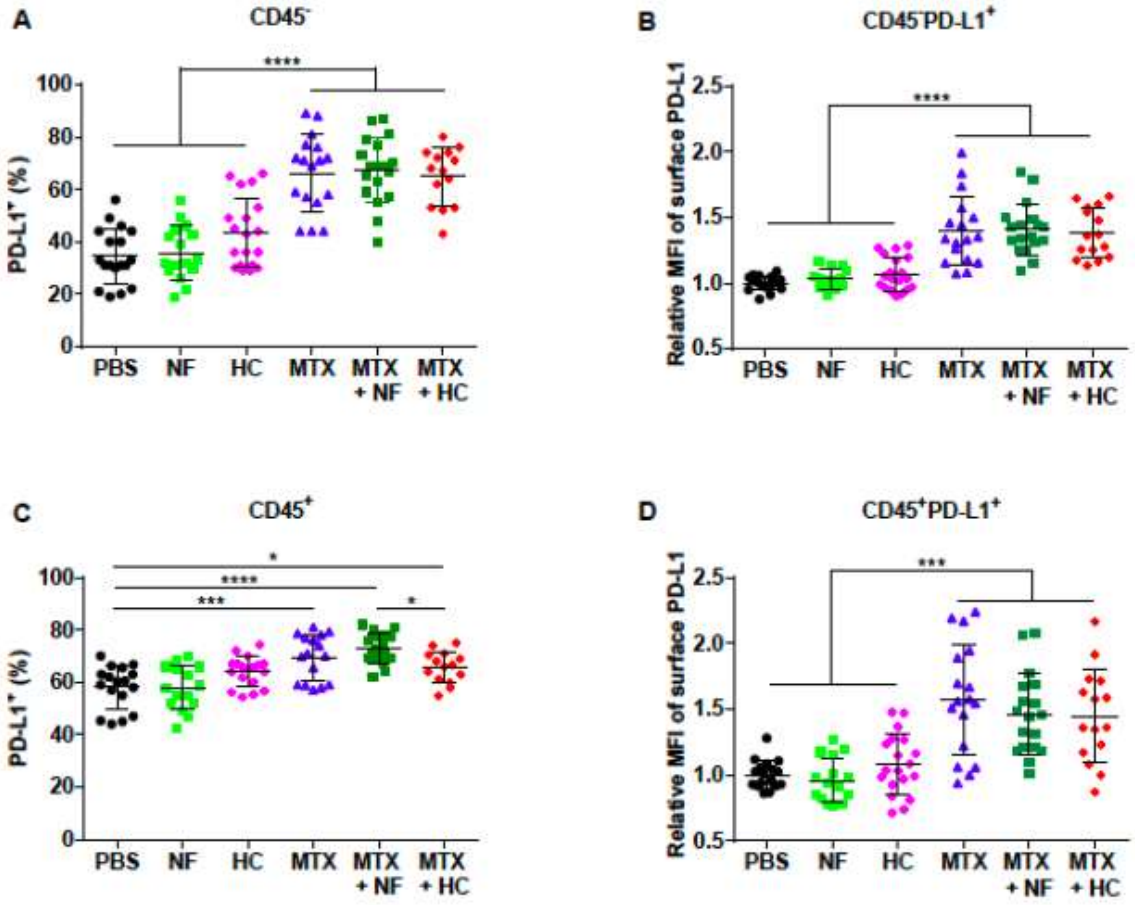


Champiat S, Journal of Thoracic Oncology 2014

Hallmarks of ICD: DAMPs



ICD = upregulates PD-L1 on tumor cells + leukocytes



Kroemer, ESMO 2019 unpublished

Immunogenic Cell Death: can we monitor tumor cell death?

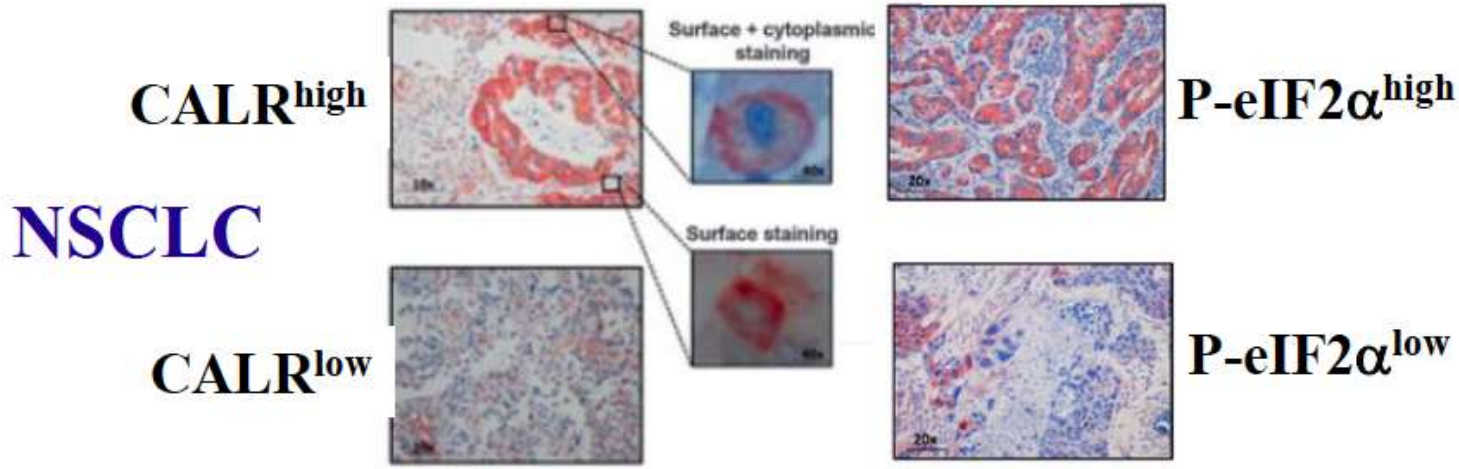
Two Major pre-mortem cell death modality

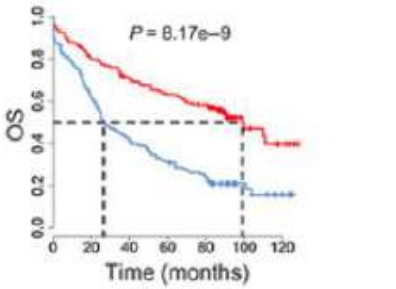
- Autophagy with lysosomal secretion of ATP
- ER stress and exposure ER protein on cell surface

ICD:

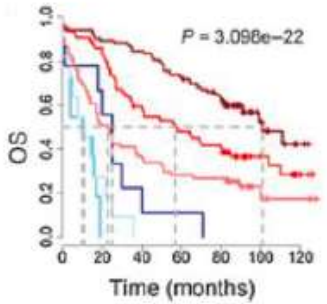
- Type I Interferon
- Release Annexin from cytoplasm
- Exodus high molecular group 1 (HMGB1) from nuclei

Markers of ICD and Authophagy correlate with overall survival in NSCLC

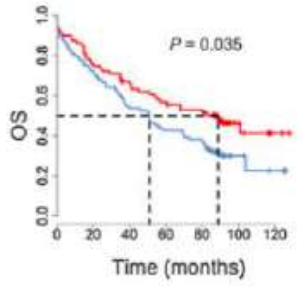




Time (months)	0	20	40	60	80	100	120
CRT High	134	107	94	83	76	19	5
CRT Low	136	85	58	43	33	8	3



Time (months)	0	20	40	60	80	100	120
CRT^{Hi}, stage I	103	92	87	75	64	13	3
CRT^{Hi}, stage II	64	55	37	30	26	10	3
CRT^{Hi}, stage III–IV	72	36	26	20	19	4	2
CRT^{Lo}, stage I	9	6	2	1	0	0	0
CRT^{Lo}, stage II	11	0	0	0	0	0	0
CRT^{Lo}, stage III–IV	11	3	0	0	0	0	0



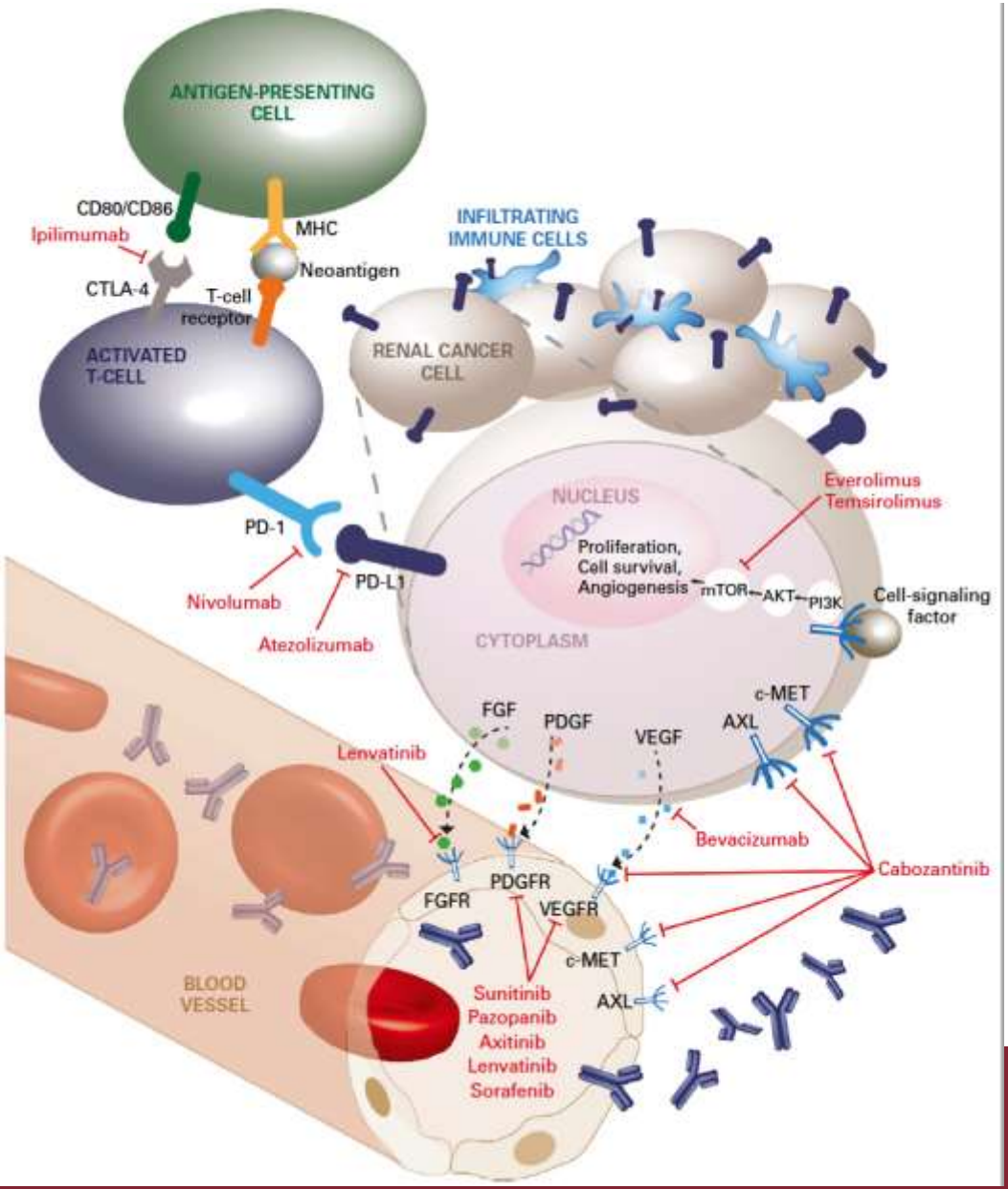
Time (months)	0	20	40	60	80	100	120
P-el2α High	80	60	52	44	40	10	2
P-el2α Low	84	61	46	36	32	5	2

TKIs and IMMUNE ACTIVATION

New vision to define resistance to ICI: what if it is mostly mediated by angiogenesis?

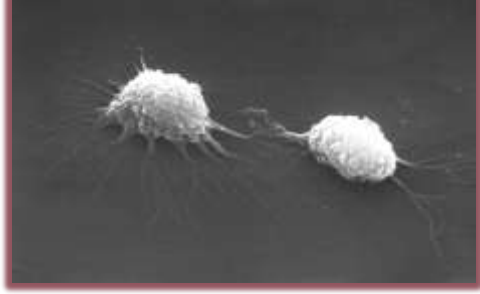
- lack of *effectors* TILs: no T cell trafficking

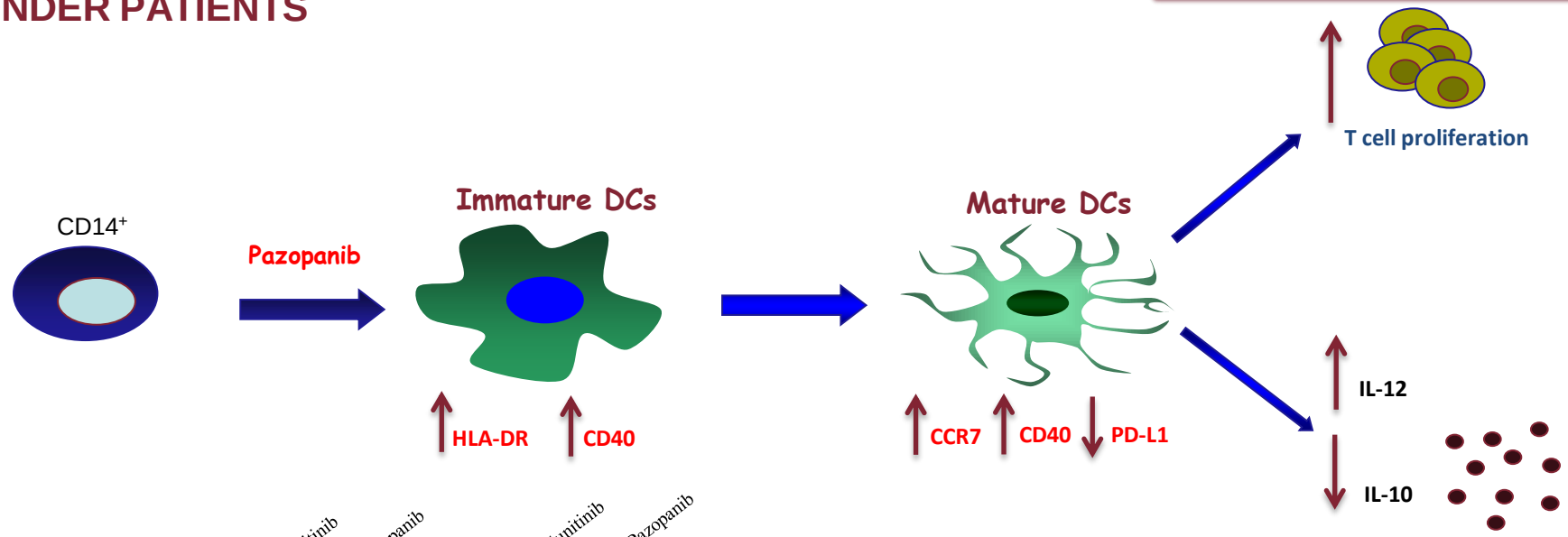
Targeting options in RCC…IMMUNE IMPACT

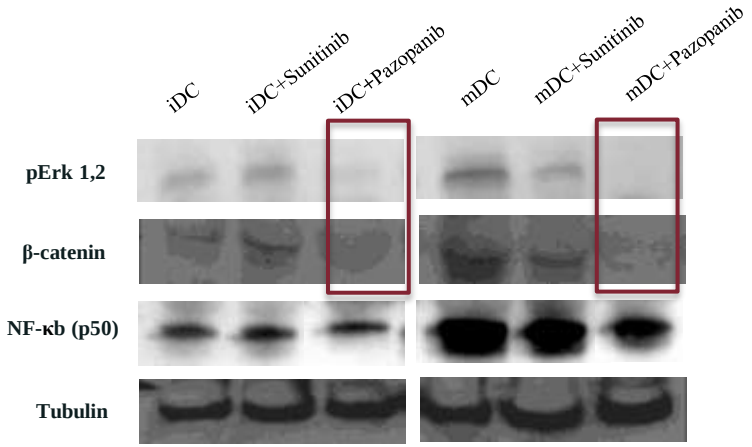


- Multitarget TKIs (suni, pazo, axi, soraf) induce tumor cell death inhibiting downstream VEGF signaling…tumor immunogenicity, ⇩ B-catenin: ⇧ lymphocyte traffic, CD137 activation
- Cabozantinib and Levantinib target more receptors (resistance) …tumor immunogenicity, ⇩ B-catenin: ⇧ lymphocyte traffic
- Rapalogs target costitutive activation of mTor path (Everolimus)
- MoAbs sequestring VEGF (bevacizumab): immunesuppression: quality DC , ⇧ lymphocyte traffic

The example of TKI Pazopanib anti VEGF-R: immunopriming effect on DC downregulating Erk/β-catenin: ACTIVATION OF T CELLS CD137+ TUMOR ASSOCIATED in RESPONDER PATIENTS







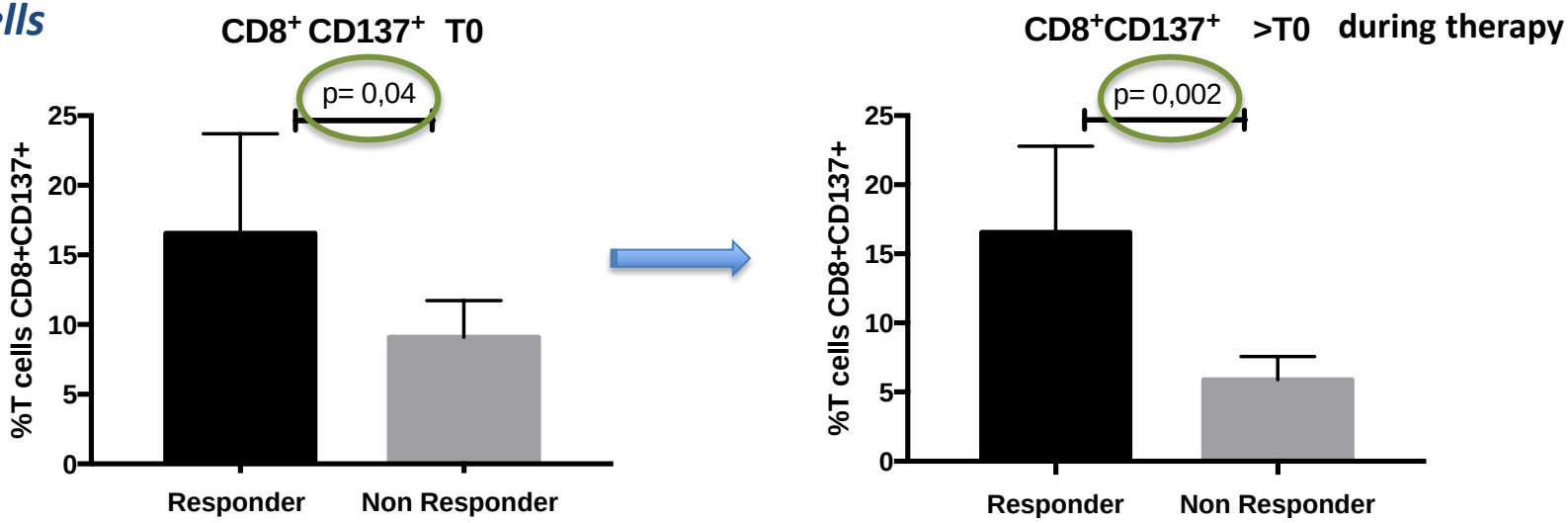
Erk/β-catenin signaling :

- **Induction of inflammatory cytokine IL-10 and IL 27**
- **Induction of IDO, Treg and TGF-β**
- **Involved in tumor progression**
- **Involved in lymphocyte trafficking**

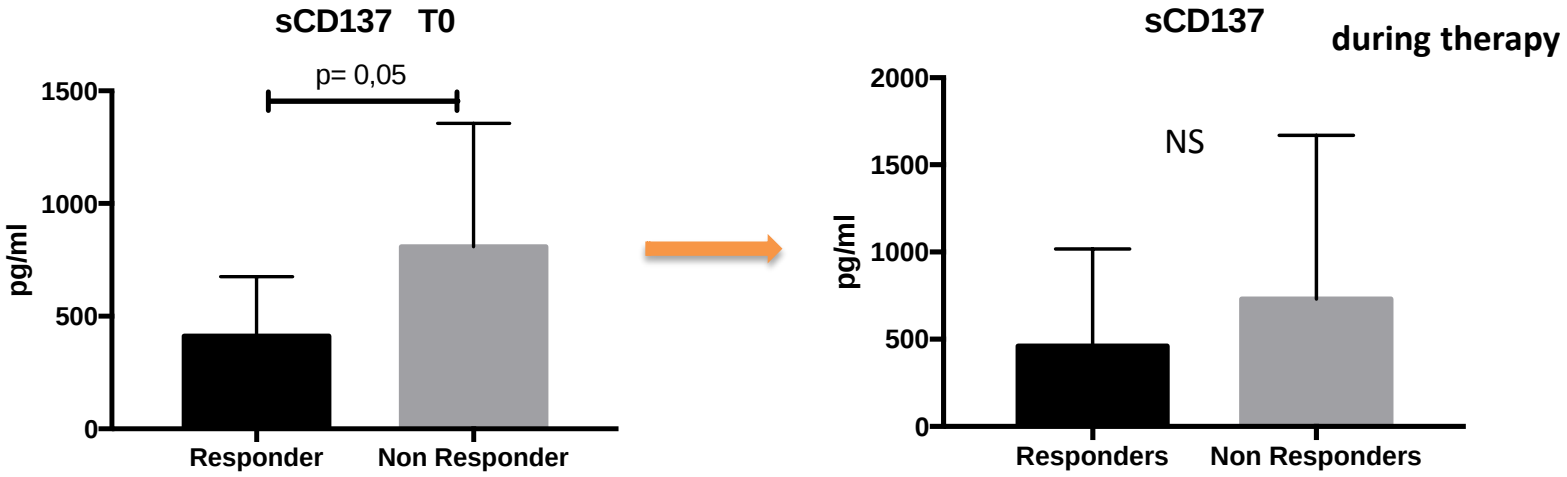
Zizzari I, Cancer Imm Res, 2018

CD137 as dynamic biomarker during TKI treatment in mRCC patients

T cells

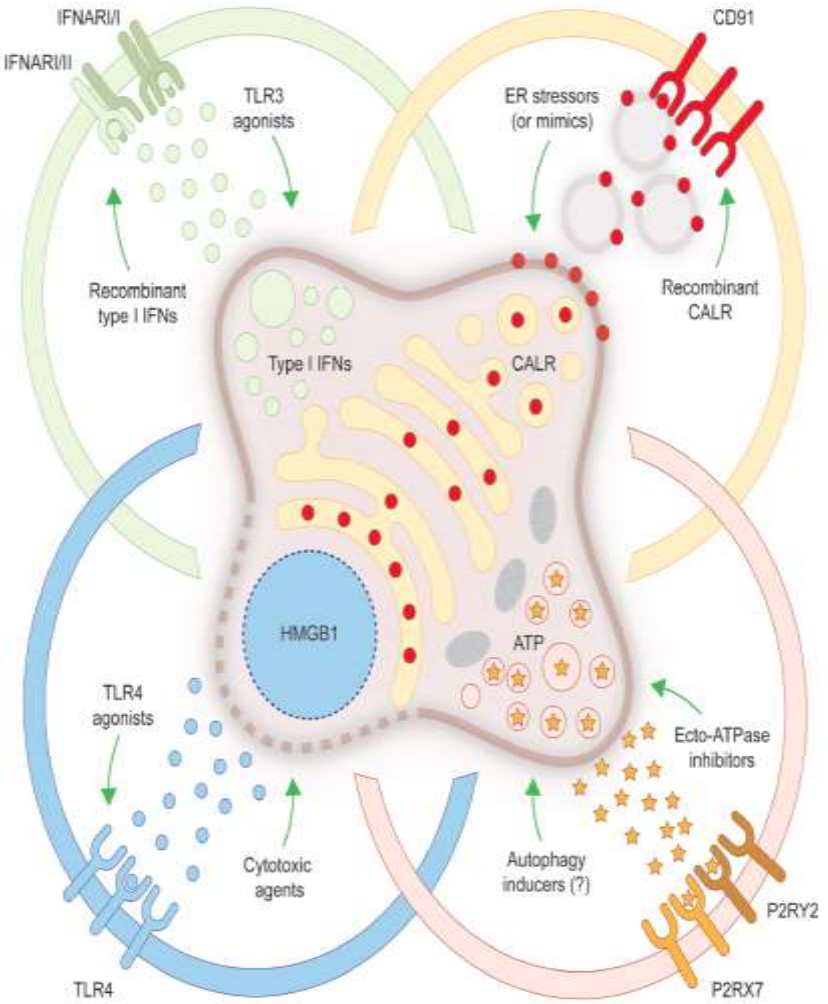


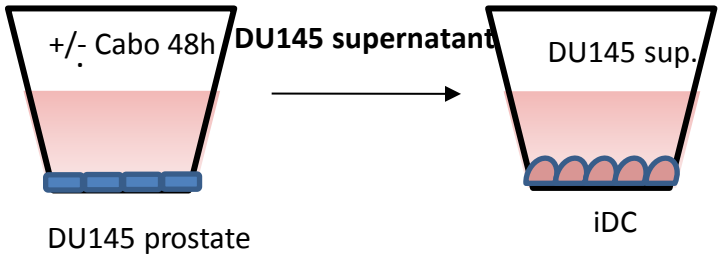
Soluble factor

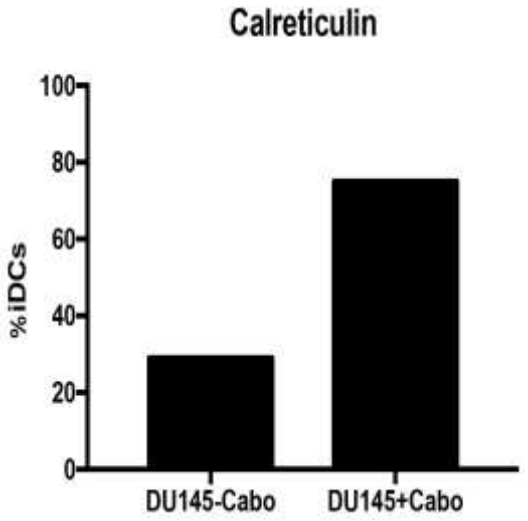


Zizzari, unpublished data

Multitarget TKI Cabozantinib induces ICD

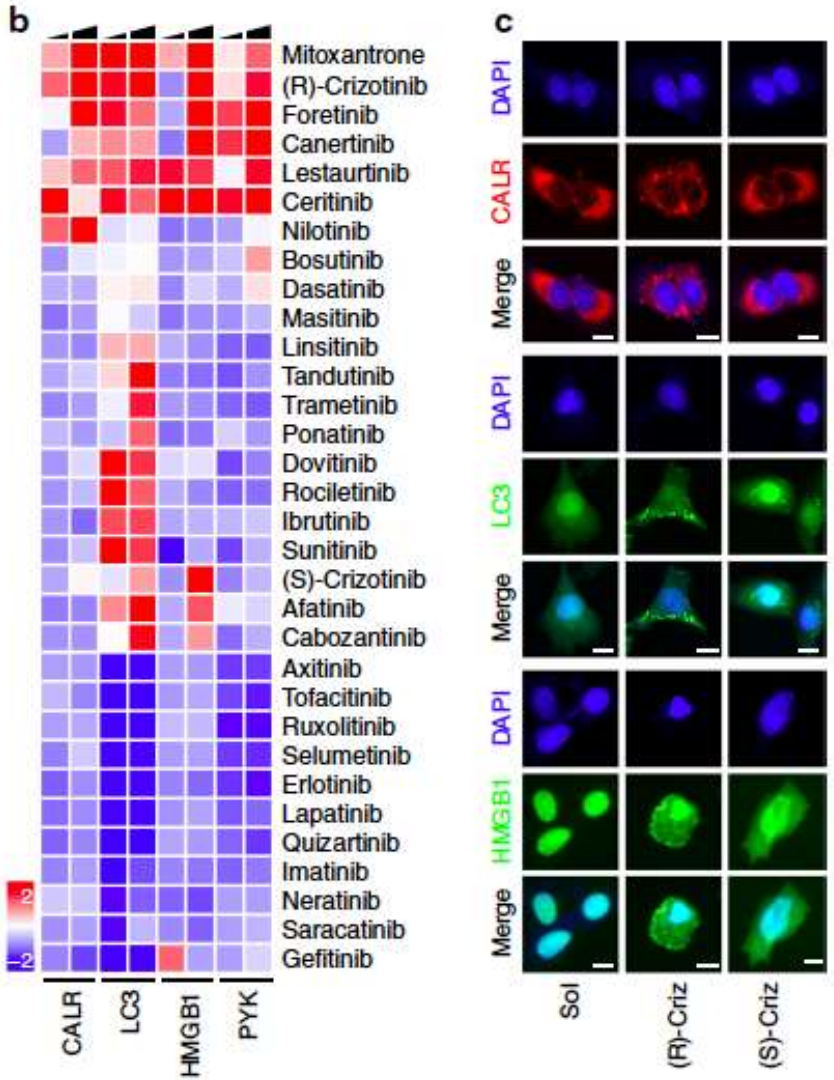




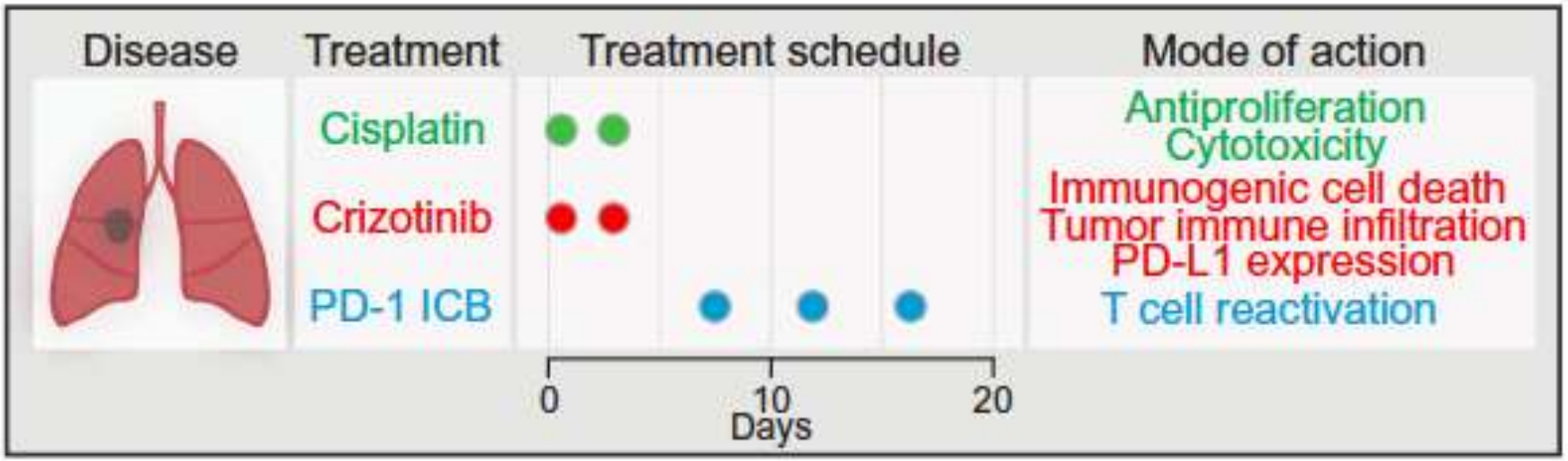


Bezu L. Front Immunol 2015

TKI assessment for induction of ICD. Calreticulin, LC3 and HMFGB1



Crizotinib TKI targeting ALK/ROS activating translocations can induce ICD as **off target** effect (action as multi-kinase inhibition)



Cisplatin and crizotinib do not induce ICD but in combination can induce ICD and sensitizes tumors to ICB

Combination treatment eradicate tumors in mice only if ICD was induced (knock out for HMGB1)

Sensitization to ICB (upregulation of PD-L1?)

The sequence cis-crizo/ICB is less toxic

Liu P et al. Nat Commun and Oncoimmunology 2019

Titan-RCC

Can IPI act as booster in nivolumab therapy in mRCC?

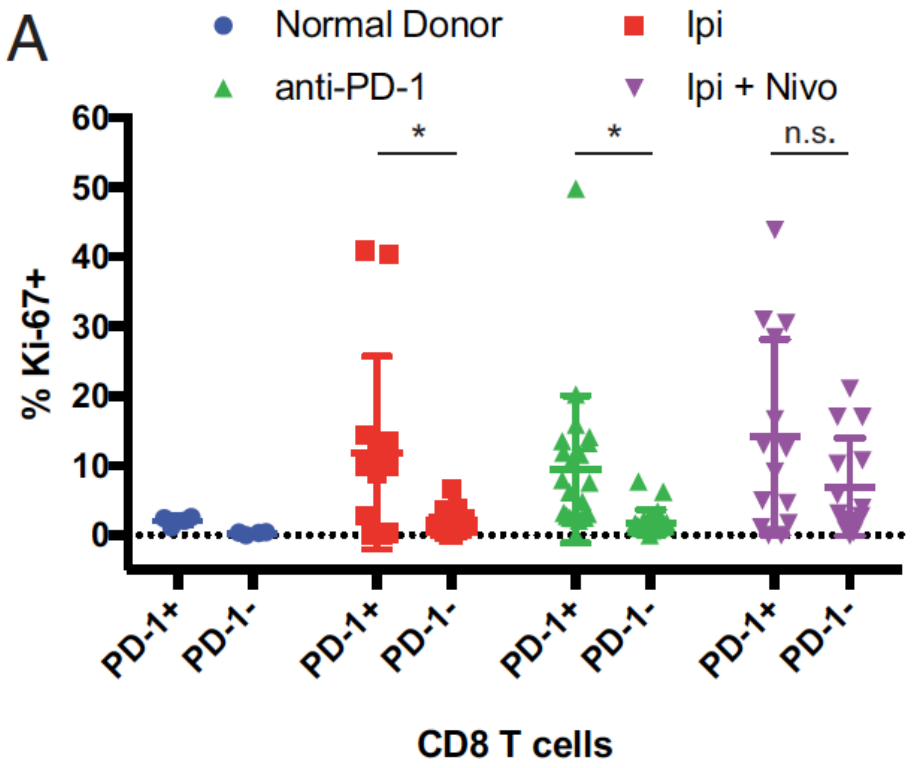
SUMMARY AND CONCLUSIONS

TITAN

TITAN-RCC

- TITAN–RCC is the first study to assess the impact of a tailored approach using ipilimumab as an immunotherapeutic boost to nivolumab monotherapy
- 1L: Boosting improved ORR [from 28.7% to 37%]
- 2L: Boosting improved ORR [from 18.2% to 28.3%]
- No new safety signals were identified
- TITAN-RCC provides further evidence to the added value of ipilimumab in combination with nivolumab in advanced RCC
- Further follow-up is ongoing to characterize duration and depth of response, and may support this innovative treatment strategy based on individual response

COMBINATION THERAPIES: IPI + NIVO a winning COMBO

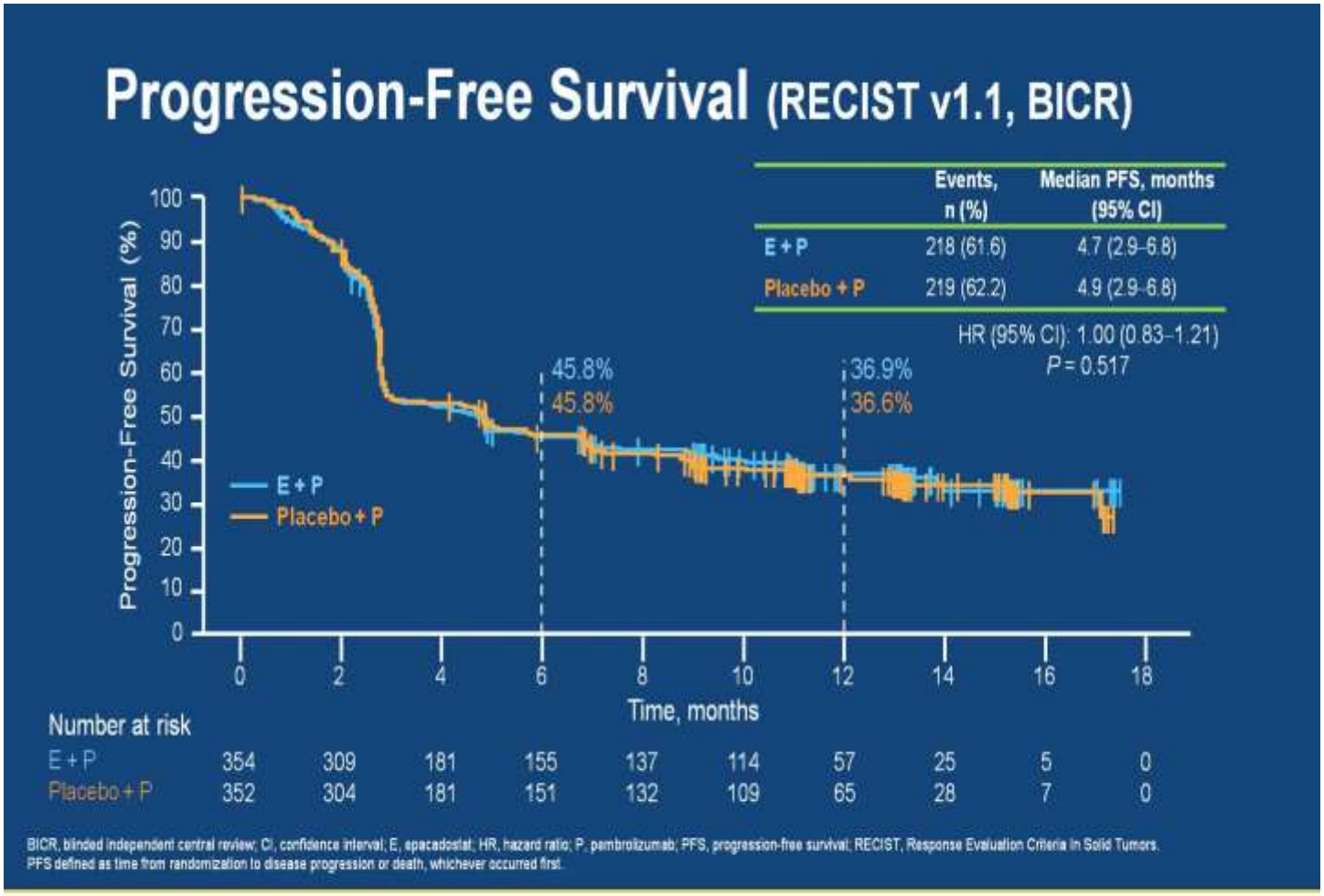


- IPI+NIVO induce proliferation of PD-1+ and PD-1- T cells
- IPI + NIVO increases frequency terminally differentiated effector CD8*
- IPI+ NIVO enhance CD4 effector function

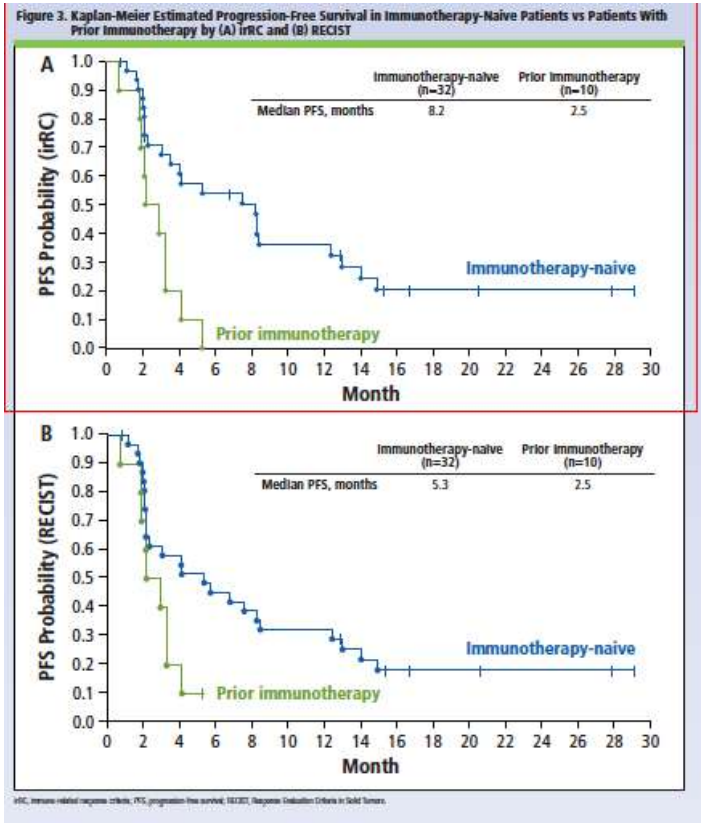
Wei SC, Pnas 2019

ASSOCIATION: warning on the immune effects of the single treatment….the disappointment of EPACADOSTAT

Epacadostat disappointment: Complete overlapping of curves

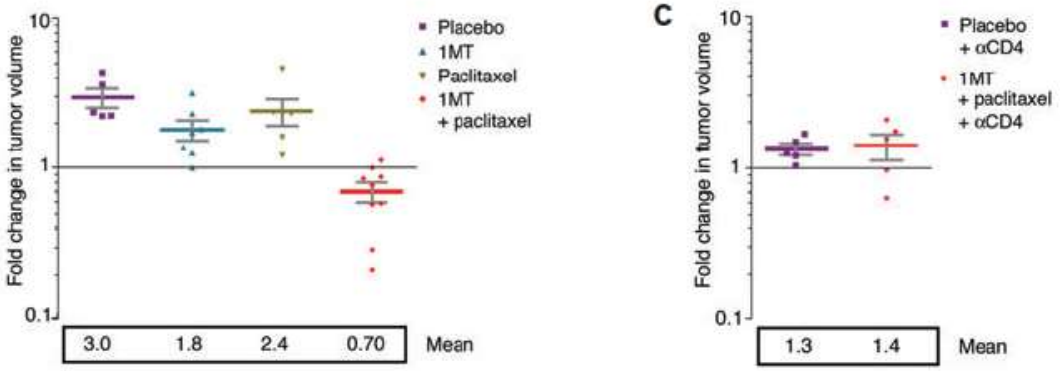


Immunotherapy naive pts appear to respond better to epacadostat (metastatic melanoma with IPI)

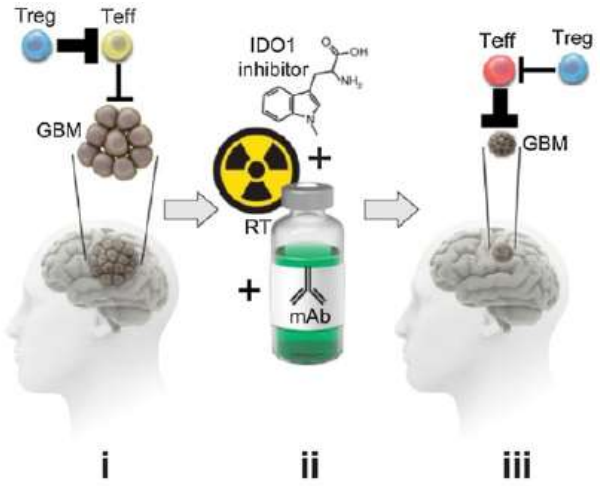


Gibney GT, ECCO-ESMO 2015

IDO inhibition (1MT) enhances efficacy of cancer chemotheraputic agents (mediated by immune cells)

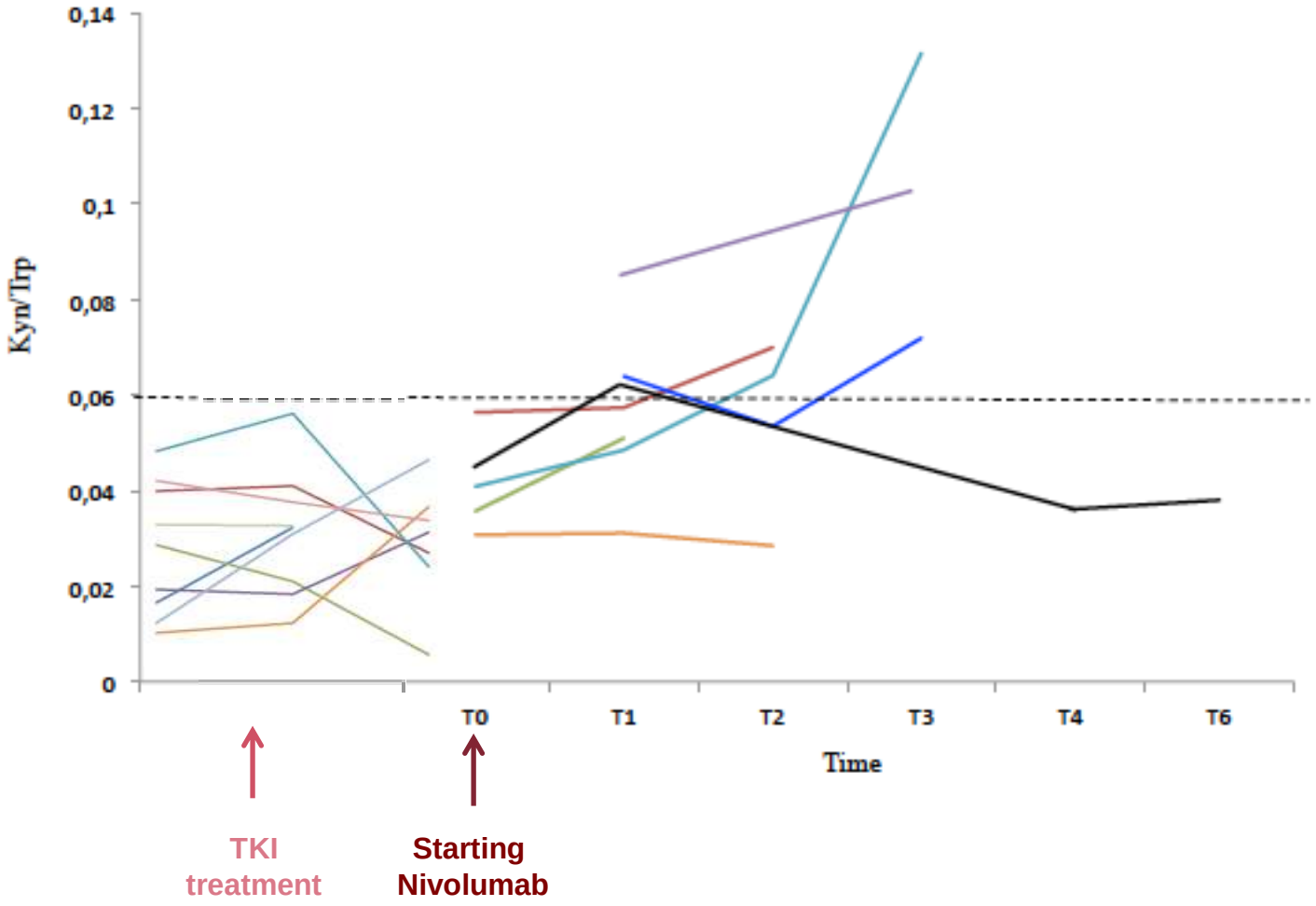


Murine model of GBM : synergy anti IDO1 and radiation and anti PD1



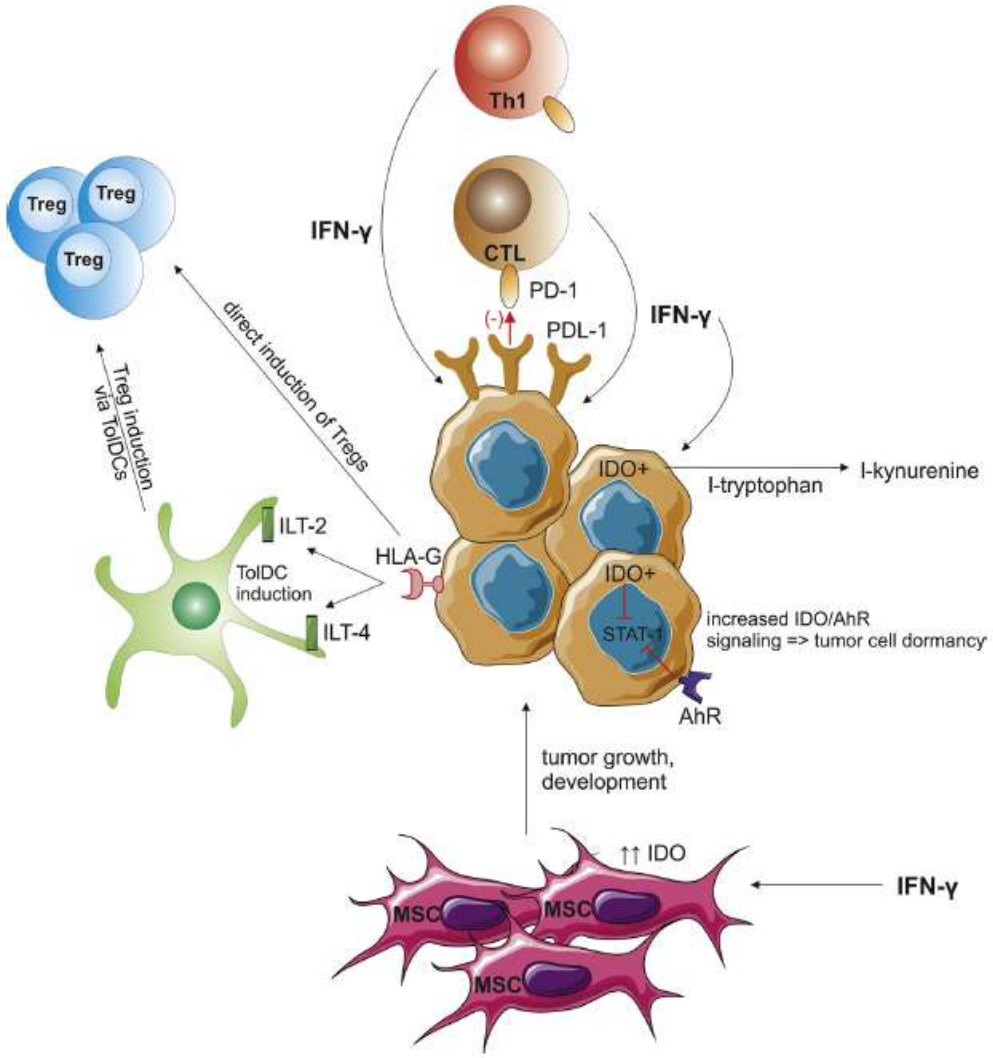
Ladomersky E, Clin cancer Res 2018

Serun Kyn/trp values in mRCC treated with TKI and at progression with nivolumab



Zizzari, unpublished

Tolerogenic role of IFN-γ in the TME



- ↑↑ **PD-L1**

↓

Suppression PD-1 T cells

- ↑↑ **IDO induction in tumor cells**

↓

Trp degradation

↓

Immunological tolerance

- ↑↑ **IDO and Aryl hydrocarbon R increased**

↓

tumor cell dormancy

Rozman P et Svajger U, Cytokine and Growth factor Rev. 2018

Possible suggestions:

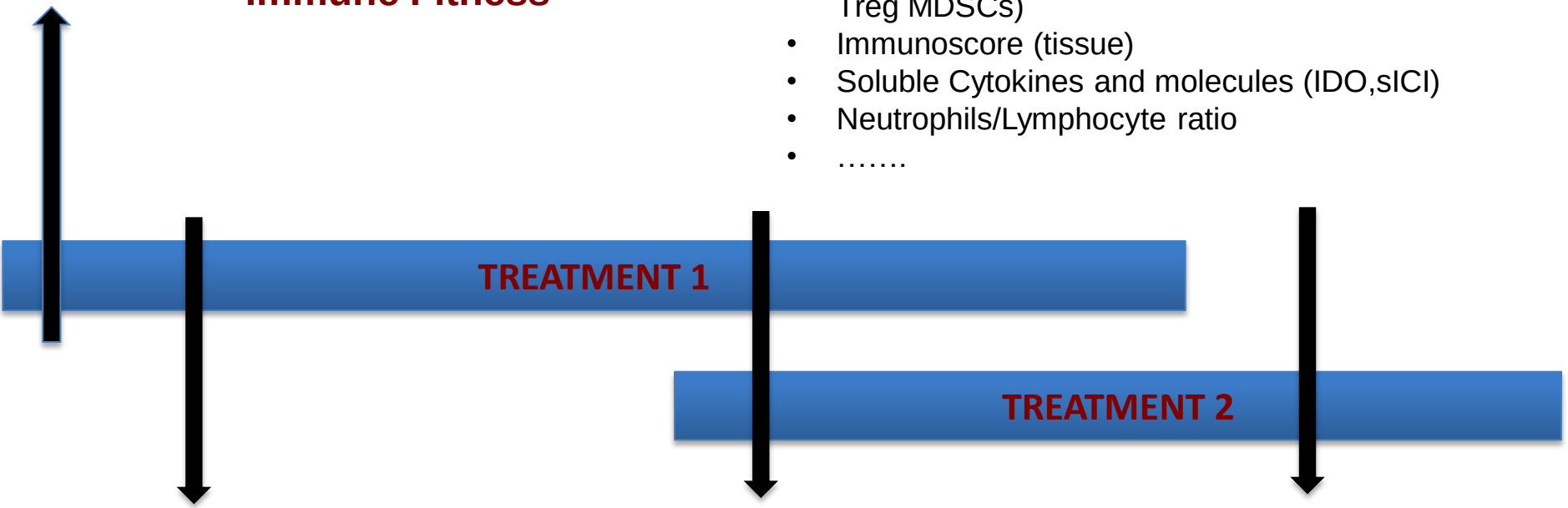
- Epacadostat in:

1. Association /alternate with chemotherapy/radiotherapy /target/TKI to limit IFN-γ production and cytokine storm (anti-PD1 therapies) followed by immunotherapy with ICI (to expand signal) : **PREPARE THE PATIENT TO RECEIVE ICI**
2. Increase dosage of epacadostat
3. Pilot proof of concept studies immunomonitored guided trials (serum IDO, kyn/trp)

Reverse translation: from bedside to bench and back again…

Immune competence of the patient: Immune Fitness

- Mutational load,microsatellite instability
- Immune Cell Profile (immunosuppression, Treg MDSCs)
- Immunoscore (tissue)
- Soluble Cytokines and molecules (IDO,sICI)
- Neutrophils/Lymphocyte ratio
- …….



Longitudinal tissue and blood samples….THE DYNAMIC MARKERS

- Immune cell profile (numbers,phenotype, ratios)
- TAM and DCs (numbers/ratio/immune profile)
- PD1/PD-L1 and CTLA4 level/other ICI
- Soluble Cytokines and molecules (IDO,sICI)
- Cell proliferation marker (Ki67)
- T cell clonality
- …….