V SIMPOSIO GETHI | 18/19 noviembre de 2019

Ilustre Colegio Oficial de Médicos de Madrid. Aula Jiménez Díaz. Madrid

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Mecanismos de resistencia, evidencia clínica y preclínica, y cómo superarlos.

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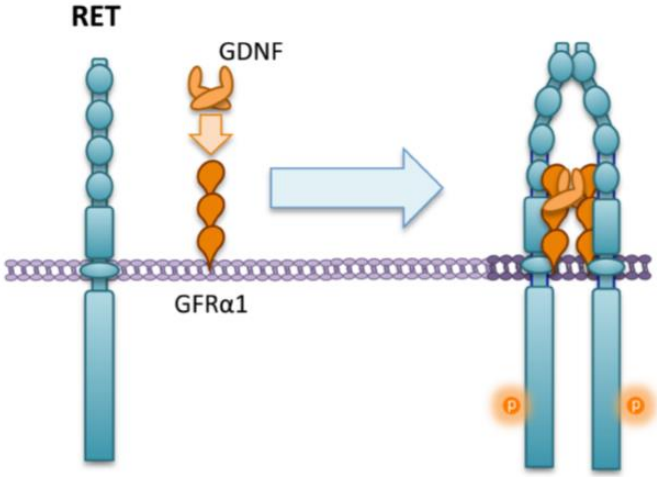
"The focus of ***precision cancer medicine is the use of patient genetic signatures to predict disease ocurrence and*** *course and tailor approaches to* ***individualized treatment*** *to improve patient outcome"*

Mulligan LM. *Endocrine-Related Cancer (2018) 25, T189-T200.*

RET → a paradigm for the power of molecular medicine cancer management.

- A tyrosine kinase receptor essential for normal development of diverse tissues (kidney, peripheral nerve lineages, neuroendocrine cells).
- RET promotes the maduration of spermatogonia, and the survival the survival and expansion of hematopoietic stem cells.

RET → broad range of functions in developing and maturing tissues.



Cadherin-like repeats

Cysteine-rich región → protein structure and ligand interation

TK domain → phosphorylation of substrates that promote multiple pathways.

Fonseca-Pereira D, et al. *Nature (2014) 514: 98-101;* Mulligan LM. *Nature Rev Cancer (2014) 14: 173-186*; Lasrado R, et al. *Science (2017) 356, 722-726*; Mulligan LM. *Endocrine-Related Cancer (2018) 25, T189-T200*;

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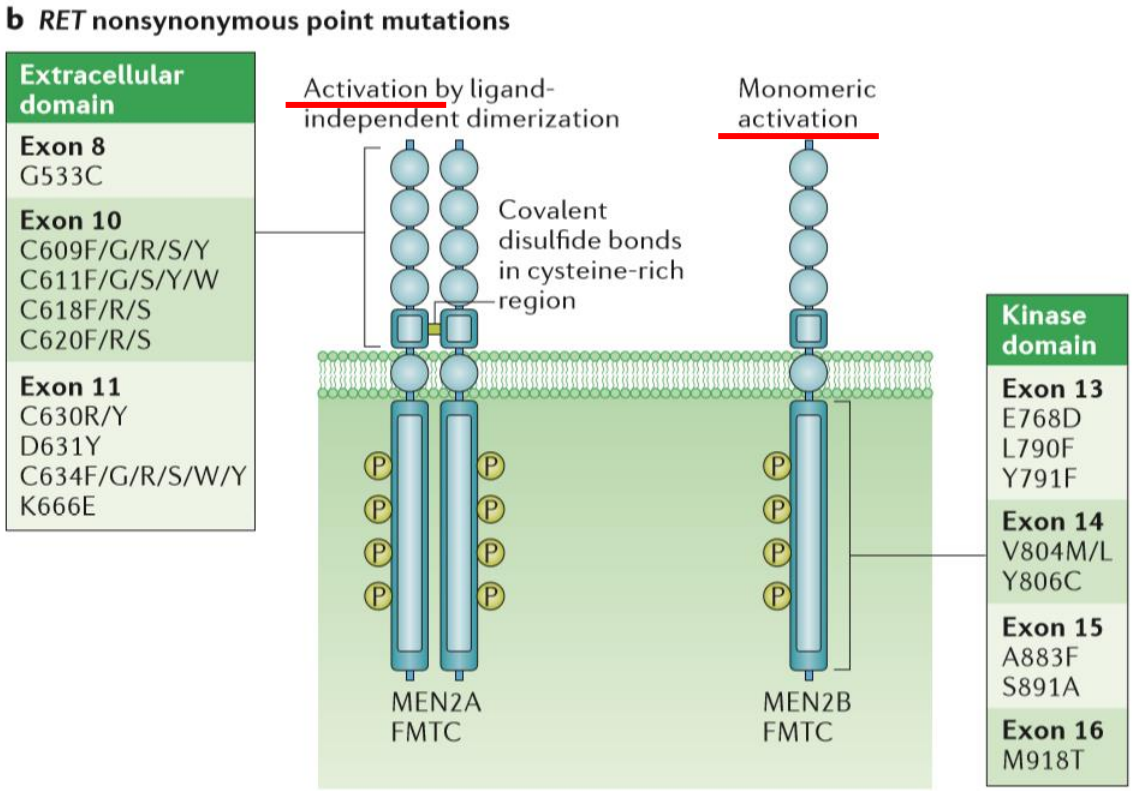
RET can contribute to oncogenesis.



1- Activating ***RET*** mutations (MEN2)

↓

The identification of the underlaying genetic cause of MEN2 has profoundly changed disease management.

Drilon A, et al. *Nat Rev Clin Oncol. 2018 Mar;15(3):151-167*;

Risk according to the specific mutation.

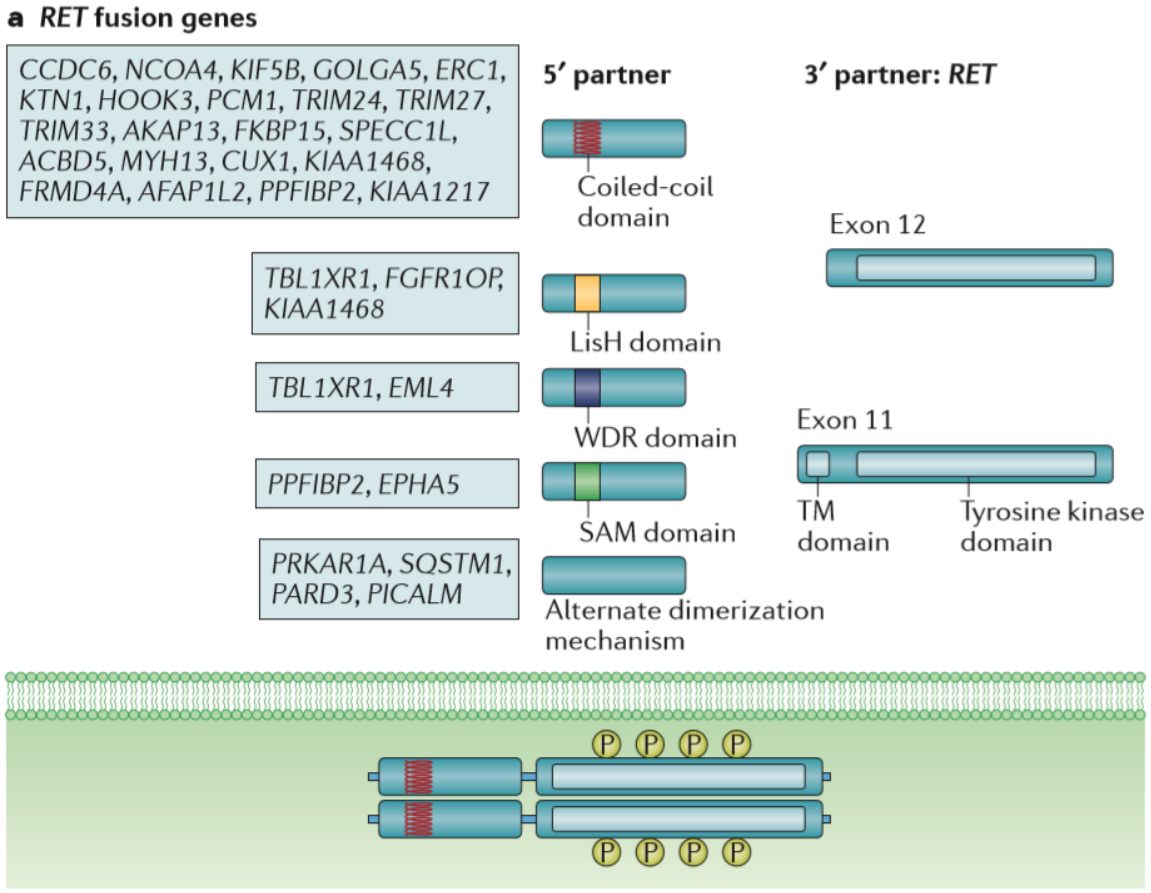
RET mutation	Exon	MTC risk level[a]	Incidence of PHEO[b]	Incidence of HPTH[b]	CLA[c]	HD[c]
G533C	8	MOD	+	-	N	N
C609F/G/R/S/Y	10	MOD	+/++	+	N	Y
C611F/G/S/Y/W	10	MOD	+/++	+	N	Y
C618F/R/S	10	MOD	+/++	+	N	Y
CF20F/R/S	10	MOD	+/++	+	N	Y
C630R/Y	11	MOD	+/++	+	N	N
D631Y	11	MOD	+++	–	N	N
C634F/G/R/S/W/Y	11	H	+++	++	Y	N
K666E	11	MOD	+	–	N	N
E768D	13	MOD	–	–	N	N
L790F	13	MOD	+	–	N	N
V804L	14	MOD	+	+	N	N
V804M	14	MOD	+	+	Y	N
A883F	15	H	+++	–	N	N
S891A	15	MOD	+	+	N	N
R912P	16	MOD	–	–	N	N
M918T	16	HST	+++	–	N	N

[a]Risk of aggressive MTC: MOD, moderate; H, high; HST, highest; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; HPTH, hyperparathyroidism; CLA, cutaneous lichen amyloidosis; HD, Hirschsprung's disease; [b]incidence of PHEO and HPTH; [c]Y, positive occurrence; N, negative occurrence: +=~10%, ++=~0–30%, +++=~5–%.

Presymptomatic genetic testing → prophylactic or early interventions before dissemination of the disease.

Samuel A Wells Jr. *Endocrine-Related Cancer (2018) 25, T1–T13*.

RET can contribute to oncogenesis.



2- somatic *RET* rearrangements

The chimeric proteins are constitutively dimerized and localized in the cytosol, enhancing pathways RET dependent.

Drilon A, et al. *Nat Rev Clin Oncol. 2018 Mar;15(3):151-167*;

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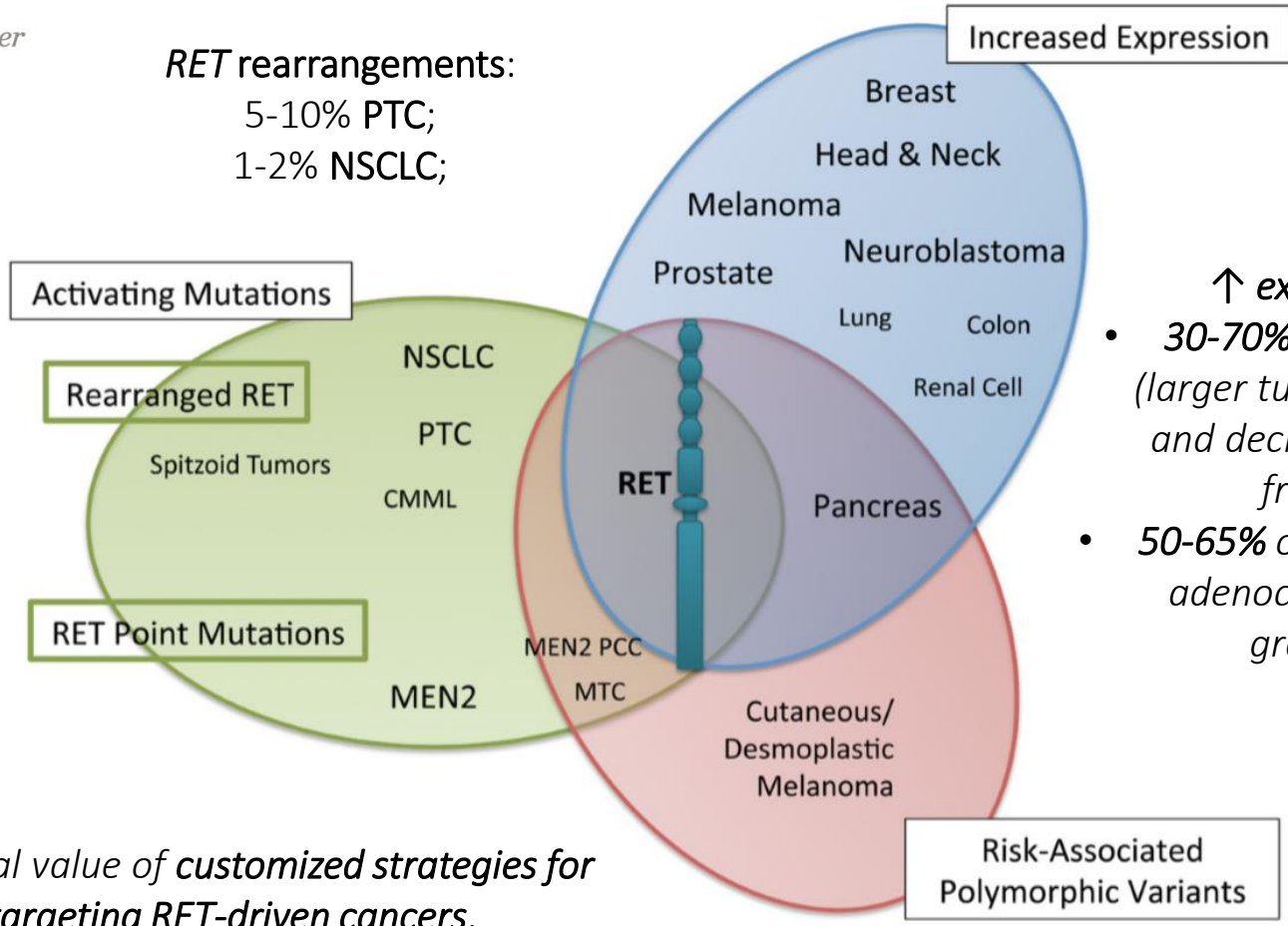
RET rearrangements:
5-10% PTC;
1-2% NSCLC;



↑ *expression in:*

- ***30-70%** of **breast cancers** (larger tumors, higher stage, and decreased metástasis-free survival).*
- ***50-65%** of pancreatic ductal adenocarcinoma (higher grade tumors).*

*Potential value of **customized strategies for targeting RET-driven cancers**.*

Stransky N, et al. *Nature Communications (2014) 5: 4846;* Antonescu CR, et al. *Am J Surg Pathol (2015) 39: 957–967*; Gautschi O, et al. *JCO (2017) 35: 1403–1410*; Mulligan LM. *Endocrine-Related Cancer (2018) 25, T189-T200*; Ferrara R, et al. *Journal of Thoracic Oncology (2018) 13: 27–45*;

There are currently **no RET-specific agents approved for clinical use**; however, **multikinases inhibitors**, originally developed to target conserved regions of other kinases have also shown **efficacy against RET**.

Mechanistically, treatment with multikinase inhibitors results in the inhibition of downstream signaling through the MAPK, PI3K, and PLCγ pathways, and decreases cell proliferation.

Takeuchi, K. et al. *Nat. Med. (2012) 18, 378–381;* **Kodama, T. et al.** *Mol. Cancer Ther. (2014) 13: 2910–2918*; **Li, G. G. et al.** Clin. Cancer Res. (2017) 23, 2981–2990**; Plenker, D. et al.** *Sci. Transl Med. (2017)9, eaah6144;* **Mulligan LM**. *Endocrine-Related Cancer (2018) 25, T189-T200*;

Clinical efficacy as *RET*-directed therapies

Table 2 Clinical trials with molecular targeted therapeutics in patients with medullary thyroid carcinoma. Reproduced from Wells SA Jr, Pacini F, Robinson BG & Santoro M, Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update, *Journal of Clinical Endocrinology and Metabolism*, 2013, volume **98**, pages 3149–3164, with permission from the Endocrine Society and Oxford University Press.

Drug (ref)	**Study**	**No. pts.**	**PR** (%)	**SD** (%)	**PFS** (mos.)
Axitinib (Cohen *et al.* 2008)	Phase II	11	18	27*	NA
Motesanib (Schlumberger *et al.* 2009)	Phase II	91	2	48**	12
Sorafenib (Lam *et al.* 2010)	Phase II	16	6.3	87.5*	17.9
Sunitinib (Ravaud *et al.* 2010)	Phase II	6	50	NA	NA
Vandetanib (Wells *et al.* 2010)	Phase II	30	20	53**	27.9#
Vandetanib (Robinson *et al.* 2010) (100 mg/day)	Phase II	19	16	53**	NA
Cabozantinib (Kurzrock *et al.* 2011)	Phase II	37	29	41**	NA
Vandetanib (Wells *et al.* 2012)	Phase III	231/100	HR 0.46	0.46 (ORR)	30.5#
Cabozantinib (Elisei *et al.* 2013)	Phase III	219/111	HR 0.28	0.28 (ORR)	11.2#
Imatinib (de Groot *et al.* 2007)	Phase II	15	0	27**	0
Imatinib (Frank-Raue *et al.* 2007)	Phase II	9	0	56*	0
Sorafenib plus Tipifarnib (Hong *et al.* 2011)	Phase II	13	38	31**	17

*>4 mos; **>6 mos; #estimated PFS in months.
HR, hazard ratio comparing PFS in treated compared to placebo control patients; mos., months; NA, not available; No. pts., number of patients; ORR, overall response rate; PFS, progression-free survival; PR, partial response (RECIST); Ref, reference; SD, stable disease.

The **experience with MTTs in the treatment of patients with advanced MTC** has been sobering. While vandetanib and cabozantinib have improved PFS, **there has been no improvement in overall survival** (**Samuel A Wells Jr.** *Endocrine-Related Cancer (2018) 25, T1–T13*)

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Vandetanib treatment in a **homogeneus series**: all children had MEN2B and the p.M918T *RET* mutation, all received the same drug → **response to therapy can vary markedly**.

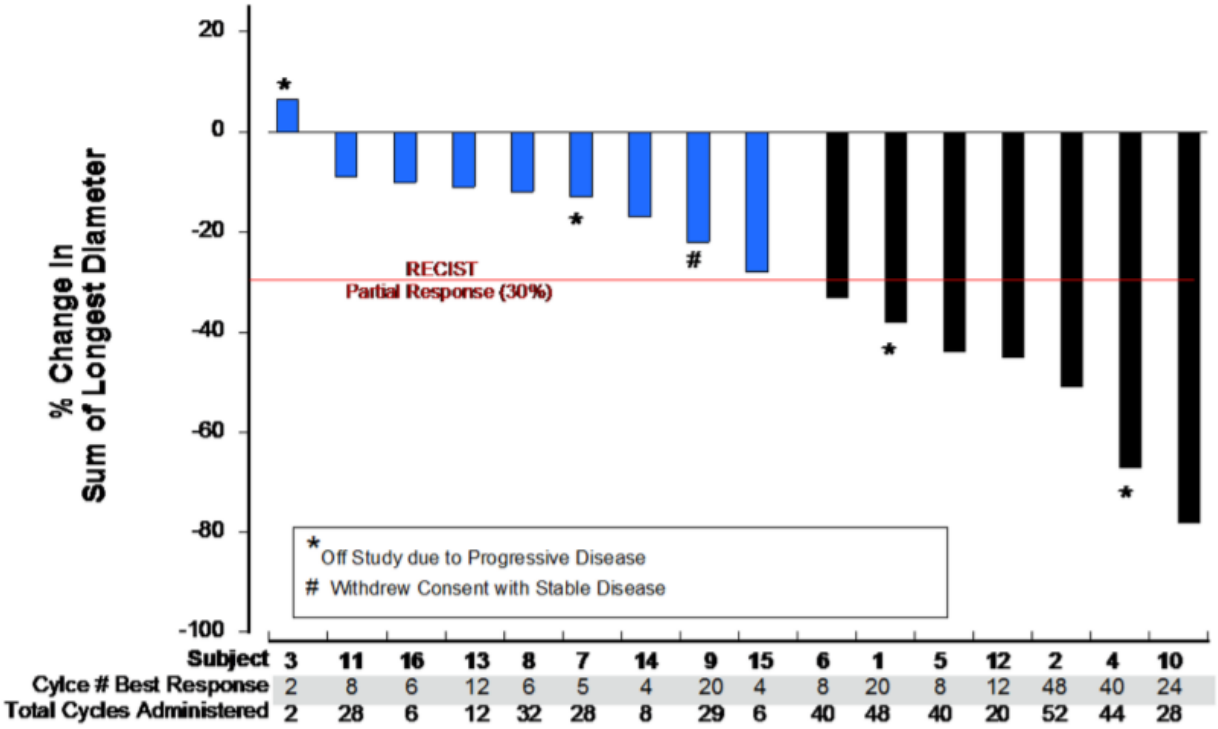

Figure 4
A waterfall plot showing reduction in size of metastatic MTC in children with MEN2B who received vanetanib. Reproduced from Fox *et al.* (2013), with permission from the American Association for Cancer Research.

The **response to a given MTT is multifactorial and depends not only on the specific target mutation**, and the treatment drug, **but on unknown factors** in the host.

Samuel A Wells Jr. *Endocrine-Related Cancer (2018) 25, T1–T13*

Possible explanations:

1- off-target inhibition.

RET kinase domain has substantial **homology** with **VEGFR2**. Many of these agents are pharmacokinetically limited in their ability to target RET compared with VEGFR2.

↓

Drug-related adverse events from off-target activity → dose-reduction and treatment discontinuation.

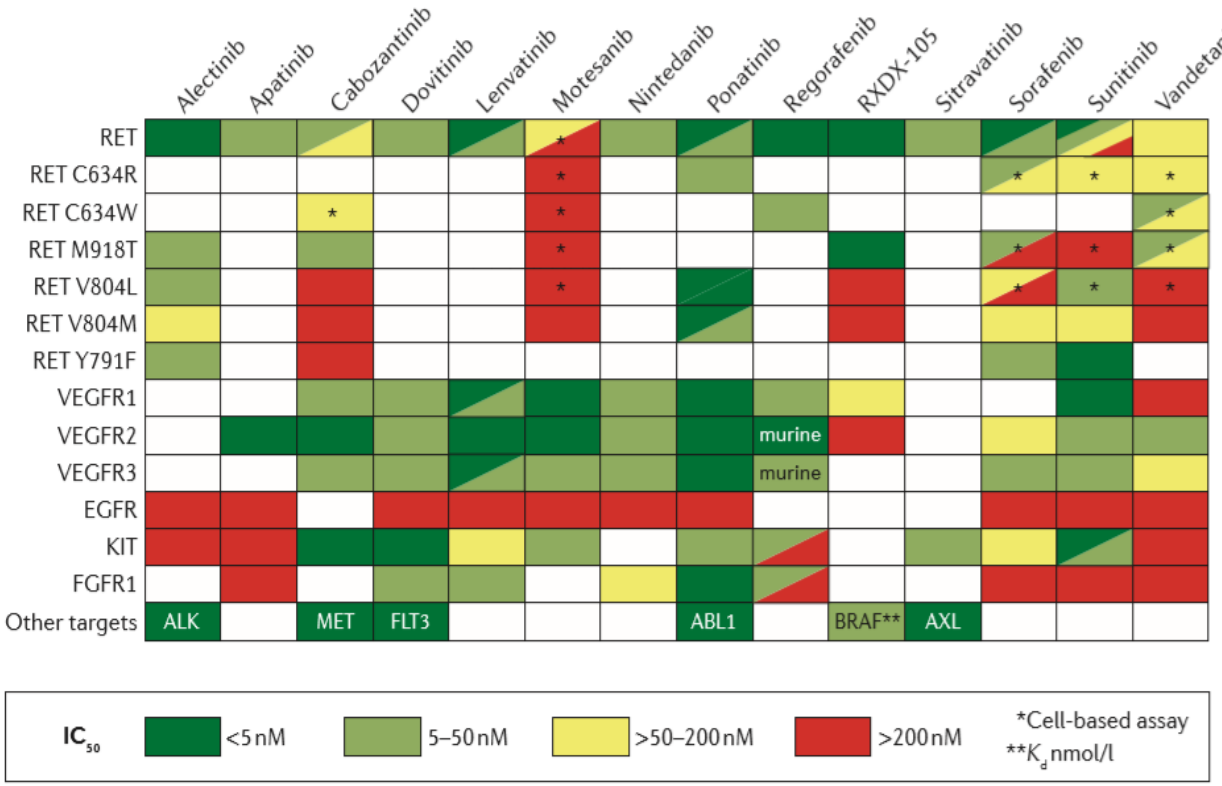

Figure 4 | **Multikinase inhibitor activity against RET and other kinases.** The half-maximal inhibitory concentration (IC_{50}) of select multikinase inhibitors with varying levels of activity against RET are shown. The various colours represent a range of IC_{50} values, from <5 nM to >200 nM. Unless otherwise indicated, the IC_{50} values shown reflect the results of *in vitro* kinase assays. The presence of two or more colours within a given box indicate different IC_{50} values reported in separate publications. A white box indicates that biochemical data are not currently available.

Drilon A, et al. *Nat Rev Clin Oncol. 2018 Mar;15(3):151-167*;

Possible explanations to understand the limited activity of MTT in *RET*-driven cancers

2- intrinsic resistance.

In NSCLC patients with *RET*-rearranged → **response dependent on the specific rearrangement** (*KIF5B–RET versus CCDC6–RET*). Preclinical data indicate a substantial increase in *RET* transcription in *KIF5B–RET* cases, possibly increasing the amount of chimeric RET oncoprotein that needs to be overcome by tyrosine-kinase inhibition.

3- Mechanisms conferring resistance.

Concurrent genomic alterations and/or bypass-pathway activation might have a role in conferring resistance. MAPK pathway reactivation via the acquisition of *KRAS* or *NRAS* mutations.

Kohno, T. et al. *Nat. Med. (2012) 18, 375–377;* Vaishnavi, A. et al. *Cancer Res. (2017) 77: 3551–3563*; Drilon A, et al. *Nat Rev Clin Oncol. 2018 Mar;15(3):151-167*;

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Other players to consider: **TME**

Human tumors → various cellular and non-cellular components (associated to cancer cells) constitute the so-called **tumor microenvironment (TME)**, which consists of extracellular matrix (ECM), mesenchymal cells (i.e., fibroblasts, pericytes, adipocytes and other stromal cells), immune-inflammatory cells, blood and lymphatic vessels.

TME and cancer cells are in close contact and **can profoundly influence each other** thus **promoting tumor initiation, progression and metastatic conversion.**

Malignant cells actively contribute **to remodel the pre-existing stroma** creating a new microenvironment that can have inflammatory or desmoplastic characteristics.

McAllister SS & Weinberg RA. *Nature Cell Biology (2014) 16: 717–727*; Sun WY et al. *Tumor Biology (2016) 37: 8197–8207*; Castellone MD, Melillo RM. *Endocr Relat Cancer. 2018 Feb;25(2):T105-T119*;

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In **thyroid cancer**, stroma composition changes among the different histotypes. An **immune/inflammatory infiltrate is associated to PTC**, while ATC, metastatic PTC and MTC display a **pronounced desmoplastic stromal reaction correlating to aggressiveness** and lymph node metastasis.

The activation of the immune-inflammatory transcriptional program depends on the signaling pathway activated and thus, on the mutation.

Castellone MD, Melillo RM. *Endocr Relat Cancer. 2018 Feb;25(2):T105-T119*;

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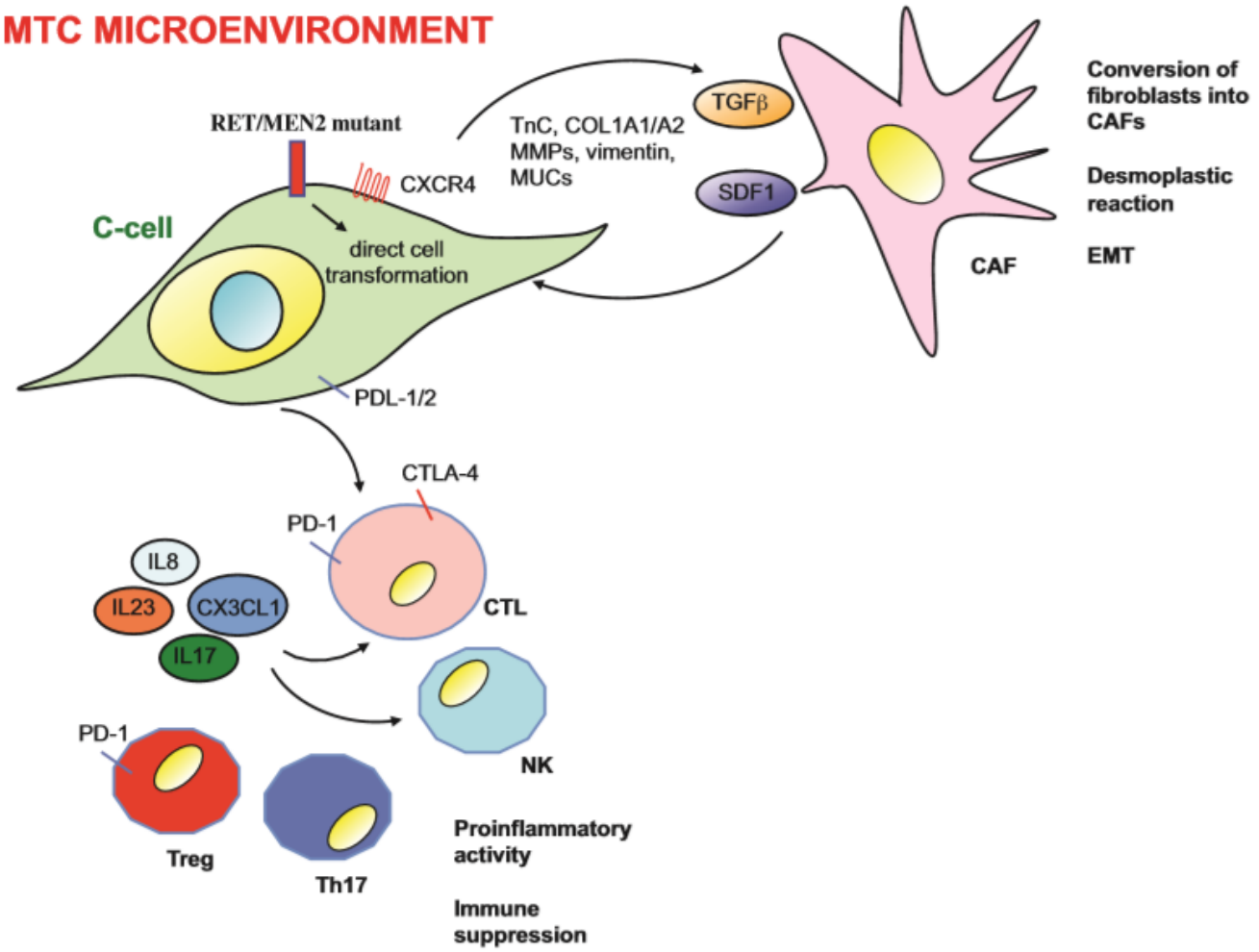

Figure 2
RET MEN2-associated mutants shape tumor microenvironment in MTC. Activation of RET in MEN2 results not only in the oncogenic conversion of the C-cell, but also in the production of factors that influence tumor microenvironment. RET-transformed C-cells can produce Tenascin C (TnC), collagens (COL1A1/2), vimentin, matrix metalloproteases (MMps) and cytokines (SDF-1, TGFβ). These factors induce fibroblast conversion into myofibroblasts, also defined as cancer-activated fibroblasts (CAFs), desmoplastic reaction and the epithelial-to-mesenchymal transition (EMT). Activated RET in the context of MEN2-associarted MTC can induce the production of immune-inflammatory molecules that can recruit and activate immune cells. CX3CL1 is induced by RET MEN2A and is involved in the recruitment of NK and CTLs. Signaling pathways activated by RET MEN2B can induce immunosuppression by recruiting Tregs and by the expression of immunomodulatory molecules, including PD L1/2. IL23 and IL17 are expressed in MTCs and can be responsible for Th17 presence and activity into tumor site. Some factors, including osteopontin (OPN), IL8 and stromal cell-derived growth factor 1 (SDF1), can increase the malignant potential of MTC cells by autocrine activation of cognate receptors.

Castellone MD, Melillo RM. *Endocr Relat Cancer. 2018 Feb;25(2):T105-T119*;

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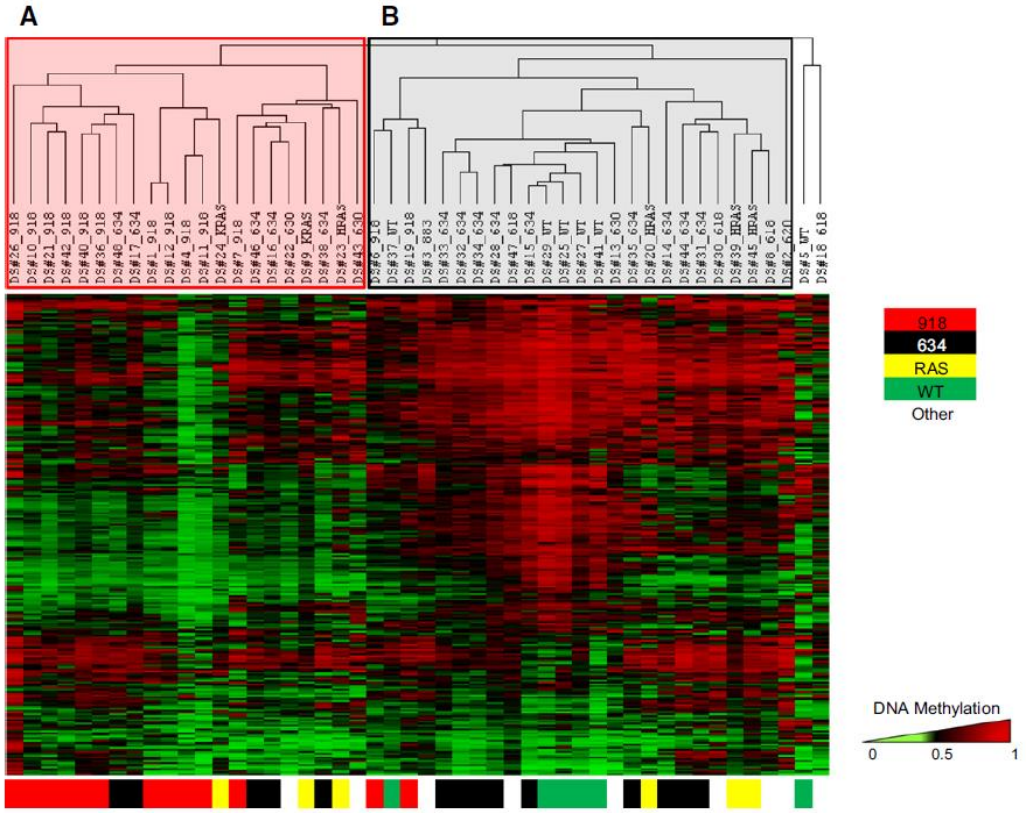
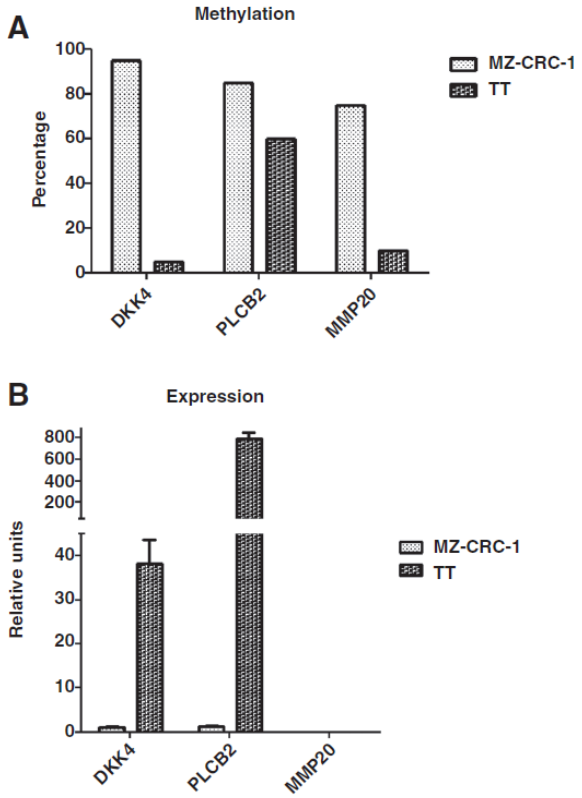
How to deal with these problems?

Combination therapy based on the biological knowledge

Based on 48 frozen MTCs genetically characterized



Integration among methylation and gene expression
→ 5% negative correlation



Mancikova V et al. *Clin Cancer Res; 2017 Mar 1;23(5):1334-1345.;* Maliszewska et al., *Am J Pathol, 2013*

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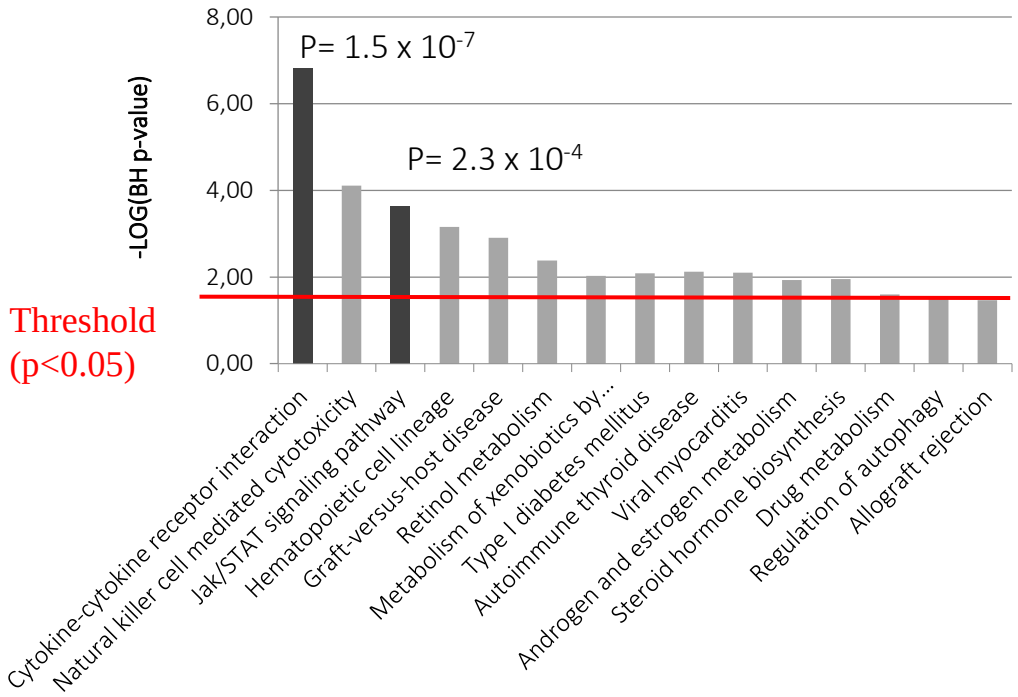
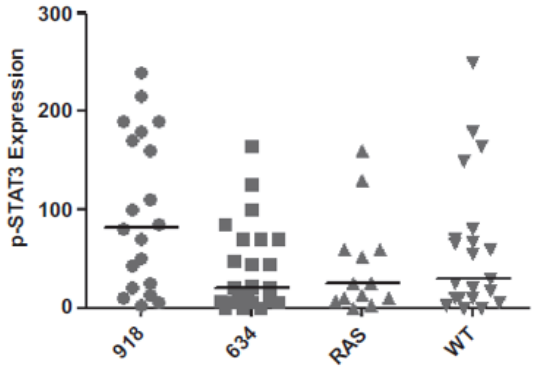
Pathway enrichment in MTC linked to p.M918T mutation

Results of DAVID functional annotation of hypomethylated genes



pSTAT3 IHC in 101 MTC.



Activation of STAT3 is associated with a **worse prognosis** due to its role in uncontrollable proliferation, inflammation and modulation of microenvironment.
Thus, STAT3 is a very **attractive molecule for targeted therapies**.

Thomas SJ, et al. *Br J Cancer 2015;113:365–71*.
Debnath B, et al. *J Med Chem 2012; 55:6645–68*.

Mancikova V et al. *Clin Cancer Res; 2017 Mar 1;23(5):1334-1345;*

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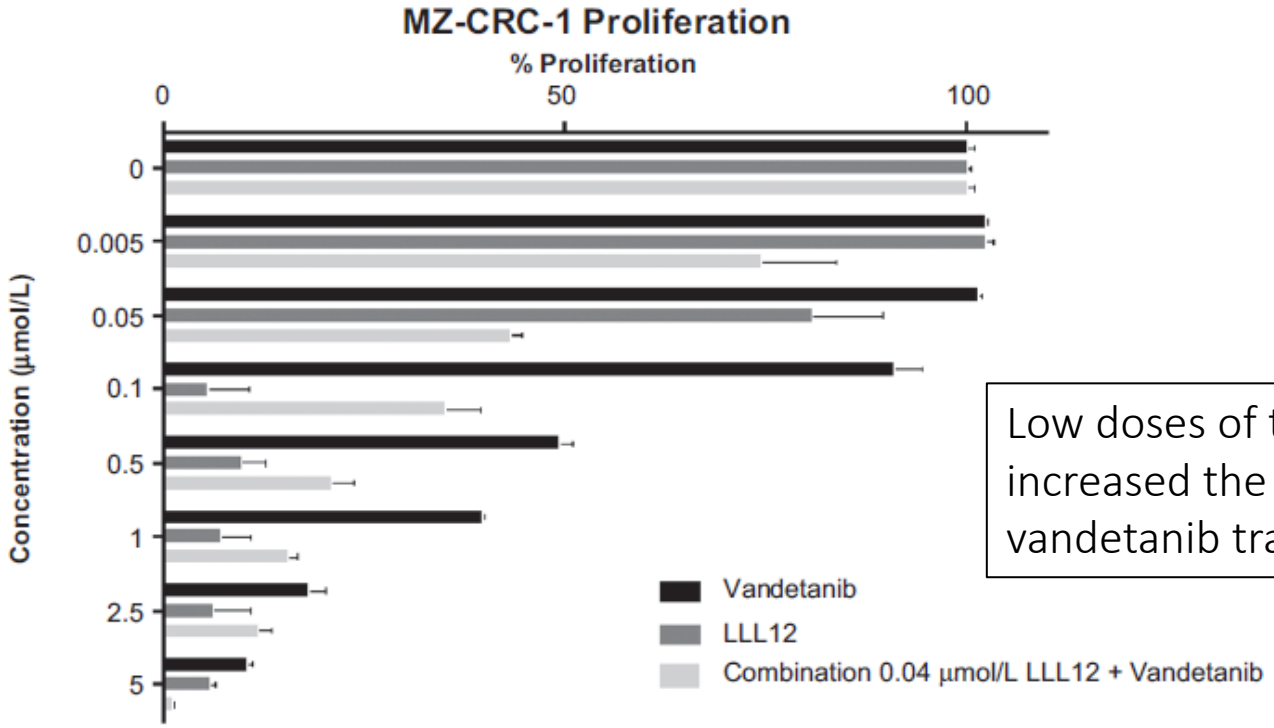
Our **aim** was **to know if this activation could be relevant for treatment response**, and then we use STAT3 inhibitors commercially available.



Low doses of the inhibitor of pSTAT3 increased the sensitivity of MTC cells to vandetanib tratment.

Mancikova V et al. *Clin Cancer Res; 2017 Mar 1;23(5):1334-1345;*

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RET-specific inhibitors → BLU-667 and LOXO-292

BLU-667. Among 40 evaluable patients, all with *RET*-altered cancers, the objective (complete and partial) **response rate was 45%.** The overall response rates were **40% and 50% among patients with MTC and NSCLC, respectively**. Most adverse events were grade 1. Tumor reductions were seen in 83% of evaluable.

LOXO-292. **Enrolled 531 patients → adverse events** occurred in ≥**15%** of patients (**most AEs were grade 1-2**). Only 9 of 531 (1.7 percent) patients discontinued LOXO-292 for treatment-related AEs. Promising results regarding durability, robust intracranial activity and safety. Objective response rate (**ORR**) -- the **calcitonin response** was 49 of 54 patients (**91%**) and the **CEA response** was 34 of 53 patients (**64%**). The **ORR** (among 26 **patients with evaluable RET fusion-positive thyroid cancer**) was **62%** (95% CI, 41-80%); 16 patients showed a response (including two PRs awaiting confirmation).

Rahal, R. et al. *Cancer Res (2016) 76 (Suppl.), 2641*; **Brandhuber, B J. et al.** Mol. Cancer Ther. (2015) 14 (Suppl. 2), B192.

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Lack of **MTKs response is multifactorial** and depends not only on the specific target mutation, and the treatment drug, but on unknown factors in the host.

Among **known factors**: 1- off-target inhibition, 2- intrinsic resistance, 3- pathway reactivation, and 4- TME.

Possible solutions: **combined treatment** (based on biological knowledge), or **more specific drugs** (to avoid off-target inhibition).

ACKNOWLEDGEMENTS

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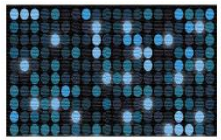
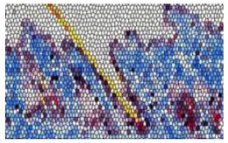

GENOMICS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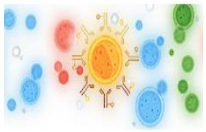
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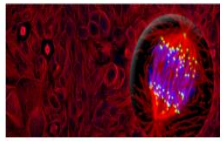
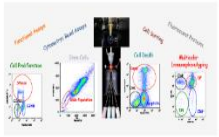

CONFOCAL MICROSCOPY


FLOW CYTOMETRY