Hypoxia normalization in tumors: the role of PTEN in tuning the microenvironment

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MNiSW

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HypoxiaNet

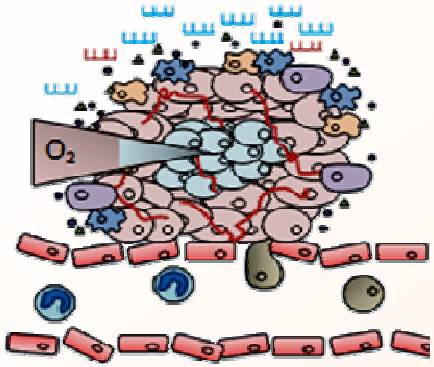
Institut de cancérologie GUSTAVE ROUSSY
VILLEJUIF - www.igr.fr

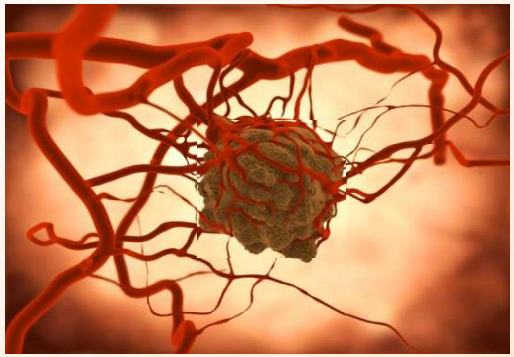
GRANOPOLE

Inserm

Gremi

Tumor microenvironment composition is ruled by hypoxia



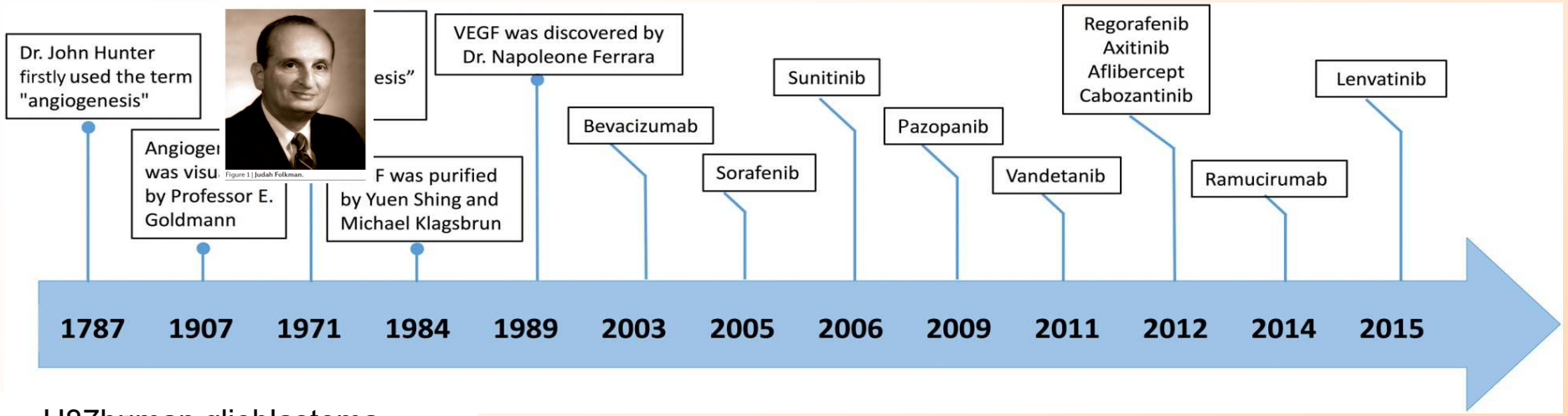


- The hypoxic stress in tumor: an angiogenesis-dependent disease

- The strategies for hypoxia alleviation:
  → vessel normalization and stabilization through PTEN

- Clinically related effects on: immunoresistance and perspectives for immunotherapy

Highlights in the development of “angiogenesis” and anti-angiogenesis drugs

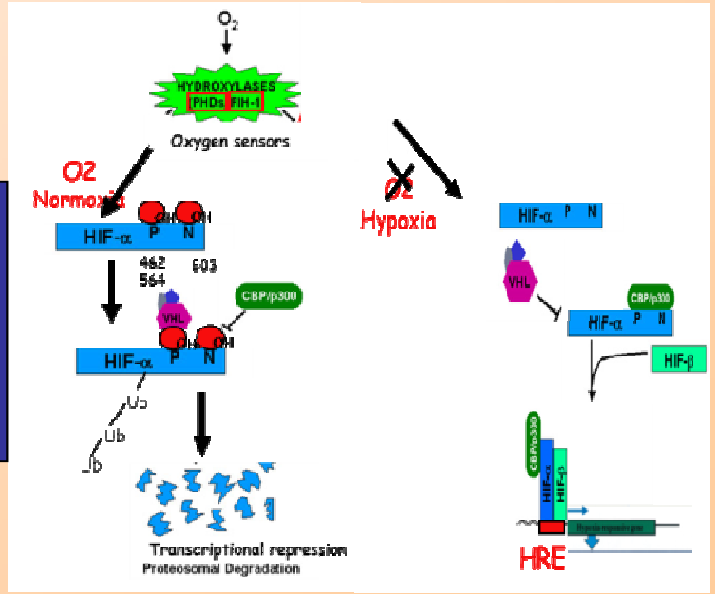


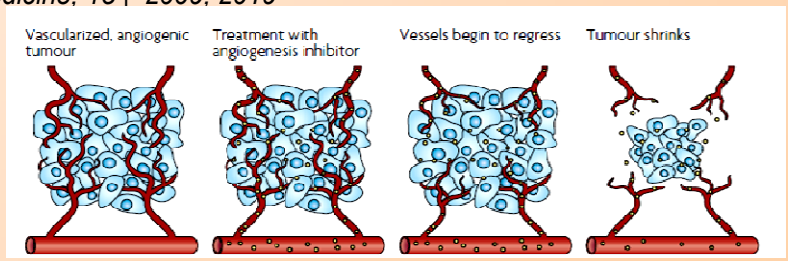
U87human glioblastoma

optical frequency domain imaging (OFDI)

Vakoc et al. nature medicine, 15 | 2009, 2019

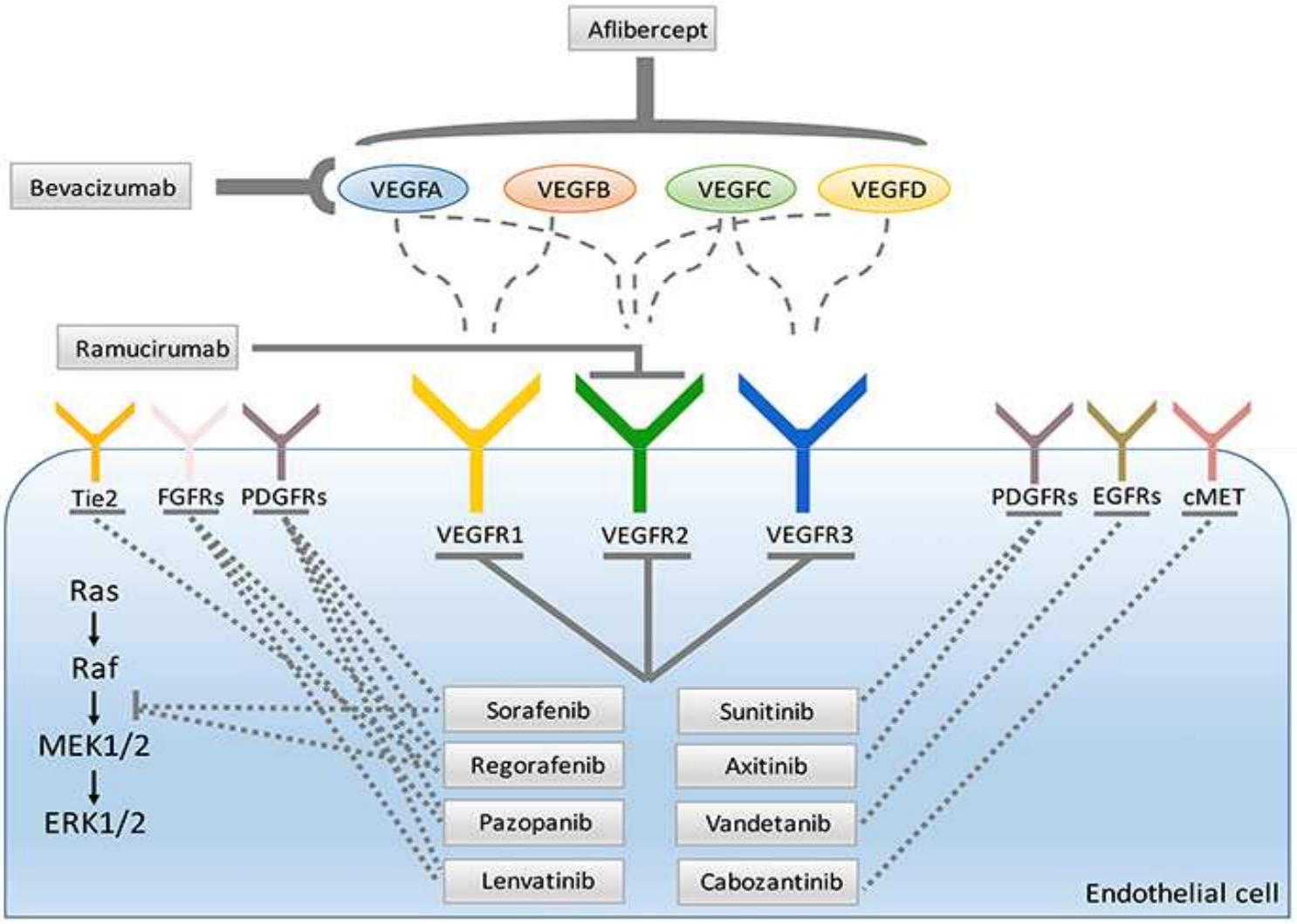
- Angiogenesis,vascular tonus and erythropoiesis (**VEGF**, **VEGF-R**, NOS2, Epo, …)
- Energy metabolism (Glut-1, Glut-3,…)
- Cellular proliferation and differentiation (TGF-β, Cyclin G2, p21…)





Results?

Main molecular targets of anti-angiogenic drugs approved for patients treatment.



Therapy for Cancer: Strategy of Combining Anti-Angiogenic and Target Therapies
Comunanza, Bussolino Front. Cell Dev. Biol., 07, 2017 https:/doi.org/10.3389/fcell.2017.00101

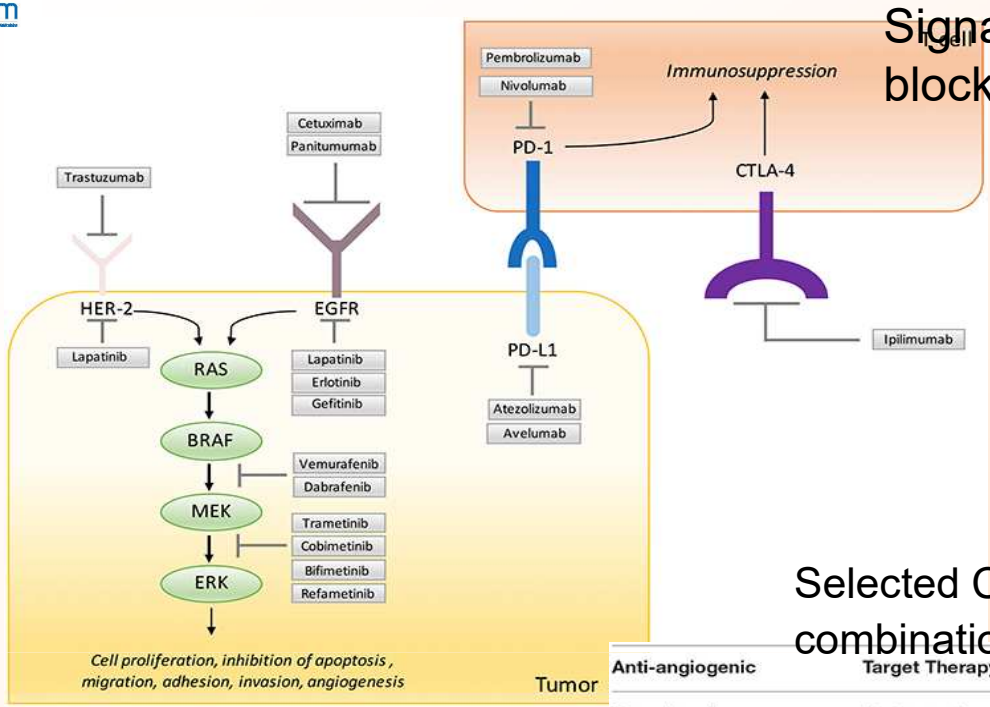
Approved VEGF-targeted therapy for oncology.

Drug	Brand name	Mechanism	Indications
Bevacizumab	Avastin (Genentech)	Monoclonal anti-VEGF antibody	CRC; NSCLC; RCC; GBM; epithelial ovarian cancer; fallopian tube cancer; primary peritoneal cancer; cervical cancer
Aflibercept	Zaltrap (Sanofi and Regeneron Pharmaceuticals)	Recombinant fusion VEGF protein	CRC
Ramucirumab	Cyramza (Eli Lilly and Company)	Monoclonal anti-VEGFR2 antibody	CRC ; NSCLC; gastric or gastroesophageal junction adenocarcinoma
Sorafenib	Nexavar (Bayer)	Multi-TKI (VEGFRs, PDGFRs, RAF, KIT, FLT3, RET)	RCC, HCC, thyroid cancer
Sunitinib	Sutent (Pfizer)	Multi-TKI (VEGFRs, PDGFRs, FLT3, CSF1R, RET)	RCC, pancreatic neuroendocrine tumors, gastrointestinal stromal tumors
Regorafenib	Stivarga (Bayer)	Multi-TKI (VEGFRs, PDGFRs, FGFRs, TIE2, KIT, RET, RAF)	GIST, CRC, HCC
Pazopanib	Votrient (GlaxoSmithKline)	Multi-TKI (VEGFRs, PDGFRs, FGFR1, c-Kit)	RCC, soft tissue sarcoma
Axitinib	Inlyta (Pfizer)	Multi-TKI (VEGFRs, PDGFRs, c-Kit)	RCC
Vandetanib	Caprelsa (AstraZeneca)	Multi-TKI (VEGFRs, EGFR, RET)	medullary thyroid cancer
Lenvatinib	Lenvima (Eisai)	Multi-TKI (VEGFRs, FGFRs, PDGFRa , RET, c-Kit)	thyroid cancer, RCC
Cabozantinib	Cometriq (Exelixis)/Cabometyx (Exelixis)	Multi-TKI (VEGFRs, cMet, AXL)	medullary thyroid cancer, RCC

CSFR1, colony stimulating factor 1 receptor; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FLT3, Fms-like tyrosine kinase 3; GBM, glioblastoma multiforme; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; KIT, stem cell factor receptor; MET, hepatocyte growth factor receptor; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; RAF, rapidly accelerated fibrosarcoma; RCC, renal cell carcinoma; RET, rearranged during transfection; VEGFR, vascular endothelial growth factor receptor.
Anti-angiogenic therapies currently approved by the US Food and Drug Administration (FDA) for the treatment of malignancies (July 2017).
For reference see http://cancer.gov.

Therapy for Cancer: Strategy of Combining Anti-Angiogenic and Target Therapies
Comunanza, Bussolino Front. Cell Dev. Biol., 07, 2017
https:/doi.org/10.3389/fcell.2017.00101

Signaling molecules and immune checkpoint blocked by targeted therapy.



Therapy for Cancer: Strategy of Combining Anti-Angiogenic and Target Therapies
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https:/doi.org/10.3389/fcell.2017.00101

Selected Clinical Trials of VEGF-targeted therapy in combination with oncogene-targeted therapy.

Anti-angiogenic	Target Therapy	Phase	Indications	ClinicalTrials.gov Identifier
Bevacizumab	Trastuzumab	2	Stage IV metastatic breast cancer	NCT00428922
Bevacizumab	Trastuzumab	3	Metastatic HER2+ breast cancer	NCT00391092
Bevacizumab	Trastuzumab	2	Breast cancer	NCT01321775
Bevacizumab	Trastuzumab	2	Metastatic HER2+ breast cancer	NCT00364611
Bevacizumab	Trastuzumab	2	Metastatic HER2+ breast cancer	NCT00670982
Bevacizumab	Trastuzumab	2	Metastatic HER2+ breast cancer	NCT00392392
Bevacizumab	Trastuzumab	2	Metastatic breast cancer	NCT00405938
Sorafenib	Trametinib	1	HCC	NCT02292173
Sorafenib	Refametinib	2	HCC	NCT01204177
Sorafenib	Refametinib	2	HCC RAS-mutated	NCT01915602
Regorafenib	Refametinib	1	Neoplasm	NCT02168777
Bevacizumab	Erlotinib	3	CRC	NCT00265824
Bevacizumab	Erlotinib	2	NSCLC EGFR-mutated	NCT01562028
Bevacizumab	Erlotinib	2	NSCLC EGFR-mutated	NCT01532089
Regorafenib	Cetuximab	1	Advanced cancers	NCT02095054
Sorafenib	Cetuximab	2	Squamos cell carcinoma of the Head and Neck	NCT00815295
Sorafenib	Cetuximab	2	CRC	NCT00326495
Bevacizumab	Trastuzumab	3	HER2-positive breast cancer	NCT00625898
Pazopanib	Lapatinib	2	HER2-positive breast cancer	NCT00558103.

CRC, colorectal cancer; EGFR, epiderma growth factor receptor; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2 ; NSCLC, non-small cell lung cancer. For reference see https://clinicaltrials.gov.

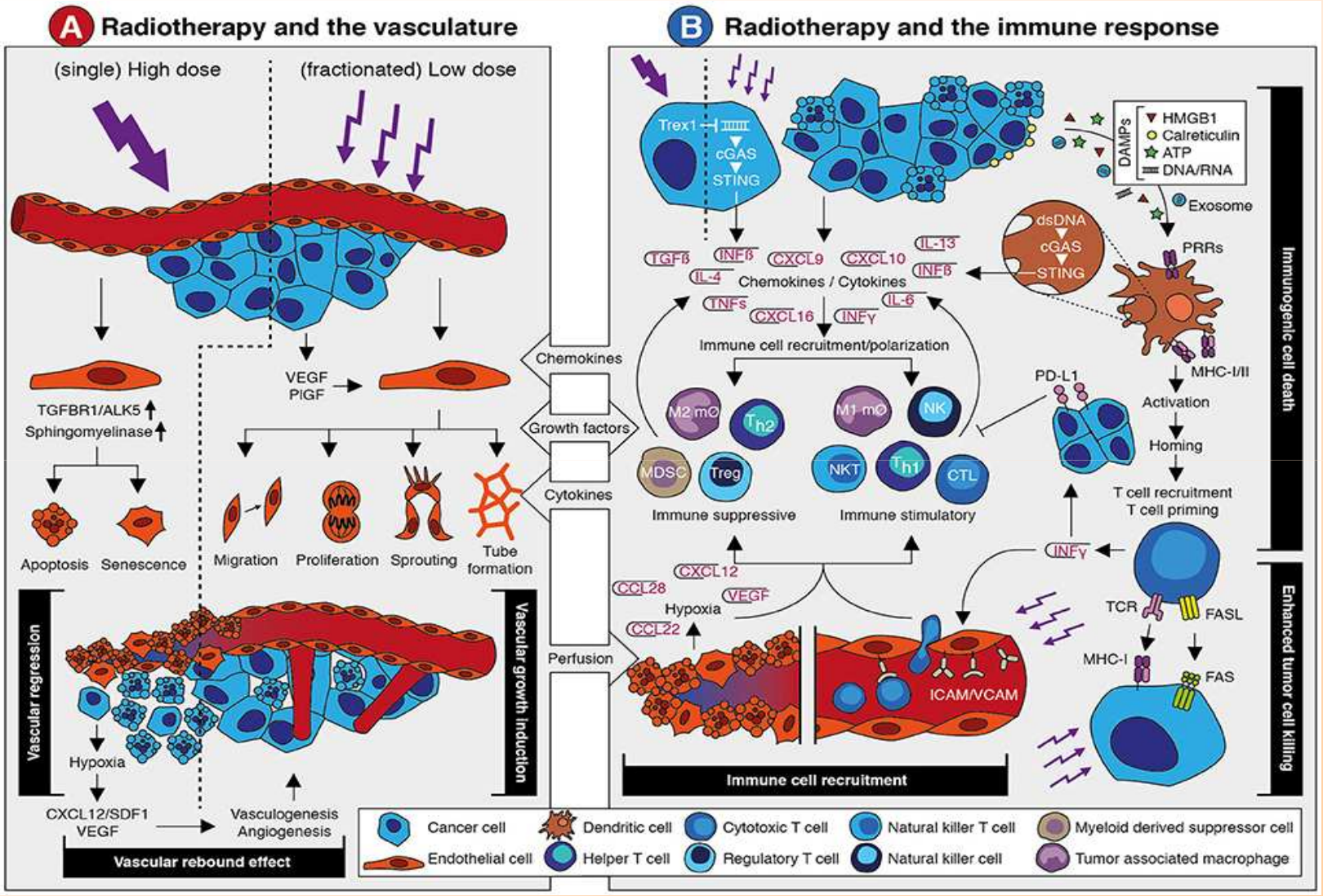
Selected Clinical Trials of VEGF-targeted therapy in combination with immune checkpoint inhibitors

Anti-angiogenic	Immunotherapy	Phase	Indications	ClinicalTrials.gov Identifier
Bevacizumab	Ipilimumab	2	Melanoma	NCT01950390
Bevacizumab	Ipilimumab	1	Melanoma	NCT00790010
Bevacizumab	Atezolizumab	2	CRC	NCT02982694
Bevacizumab	Atezolizumab	2	Melanoma brain metastases	NCT03175432
Bevacizumab	Atezolizumab	2	RCC	NCT02724878
Bevacizumab	Atezolizumab	3	RCC	NCT02420821
Bevacizumab	Nivolumab	2	Ovarian, Fallopian Tube Or Peritoneal Cancer	NCT02873962
Bevacizumab	Nivolumab	3	Glioblastoma	NCT02017717
Bevacizumab	Nivolumab	1	NSCLC	NCT01454102
Bevacizumab	Nivolumab	1	RCC	NCT02210117
Bevacizumab	Pembrolizumab	2	RCC	NCT02348008
Bevacizumab	Pembrolizumab	1/2	NSCLC	NCT02039674
Bevacizumab	Pembrolizumab	2	Glioblastoma	NCT02337491
Bevacizumab	Pembrolizumab	2	Melanoma/NSCLC brain metastases	NCT02681549
Aflibercept	Pembrolizumab	1	Solid tumors	NCT02298959
Sunitinib	Nivolumab	1	RCC	NCT01472081
Axitinib	Pembrolizumab	3	RCC	NCT02853331
Axitinib	Avelumab	3	RCC	NCT02684006
Cabozantinib	Nivolumab	3	RCC	NCT03141177

CRC, colorectal cancer; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.
For reference see https://clinicaltrials.gov.

Therapy for Cancer: Strategy of Combining Anti-Angiogenic and Target Therapies
Comunanza, Bussolino Front. Cell Dev. Biol., 07, 2017 https:/doi.org/10.3389/fcell.2017.00101

Therapeutic triad to reach adjuvant effect for radiotherapy anf imunotherapy

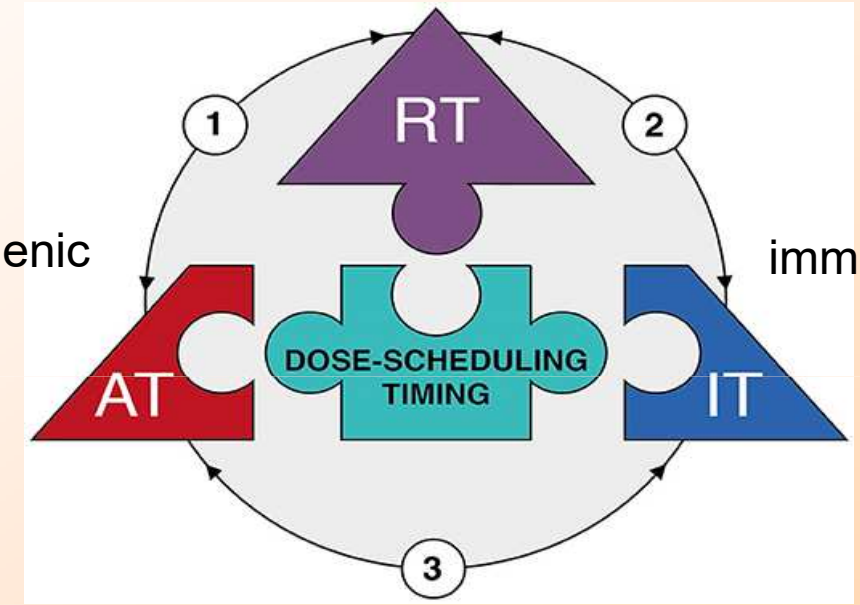


Combining Radiotherapy With Anti-angiogenic Therapy and Immunotherapy
Goedegebuure et al. Front. Immunol.,2019 doi.org/10.3389/fimmu.2018.03107

Therapeutic triad to reach adjuvant effect for radiotherapy anf imunotherapy

Radiotherapy

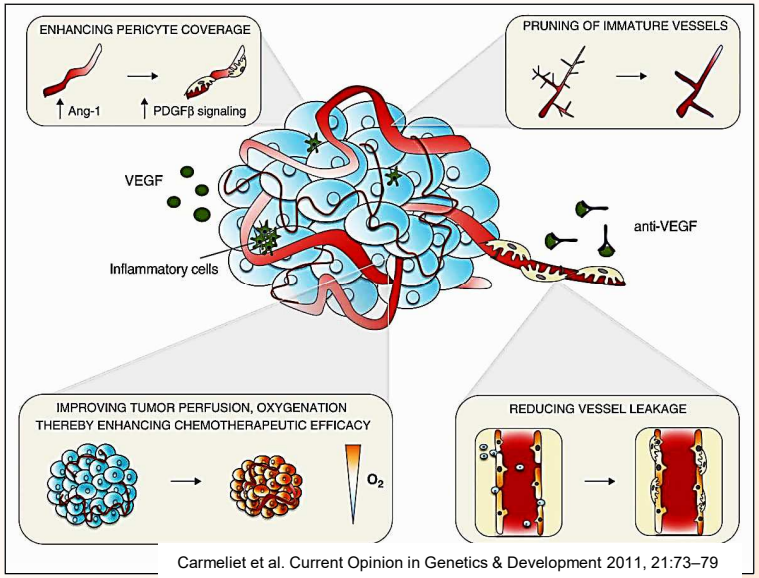
anti-angiogenic therapy



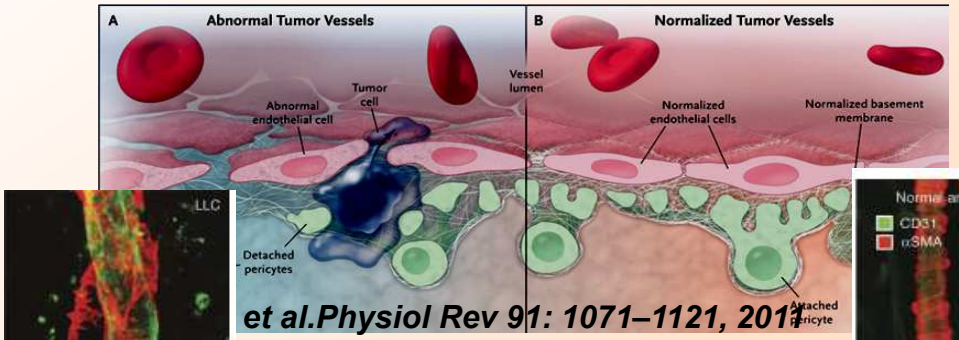
immunotherapy

Combining Radiotherapy With Anti-angiogenic Therapy and Immunotherapy
Goedegebuure et al. Front. Immunol.,2019 doi.org/10.3389/fimmu.2018.03107

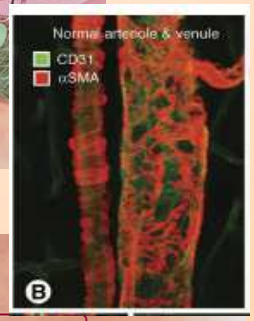
In tumors: angiogenesis normalization effects

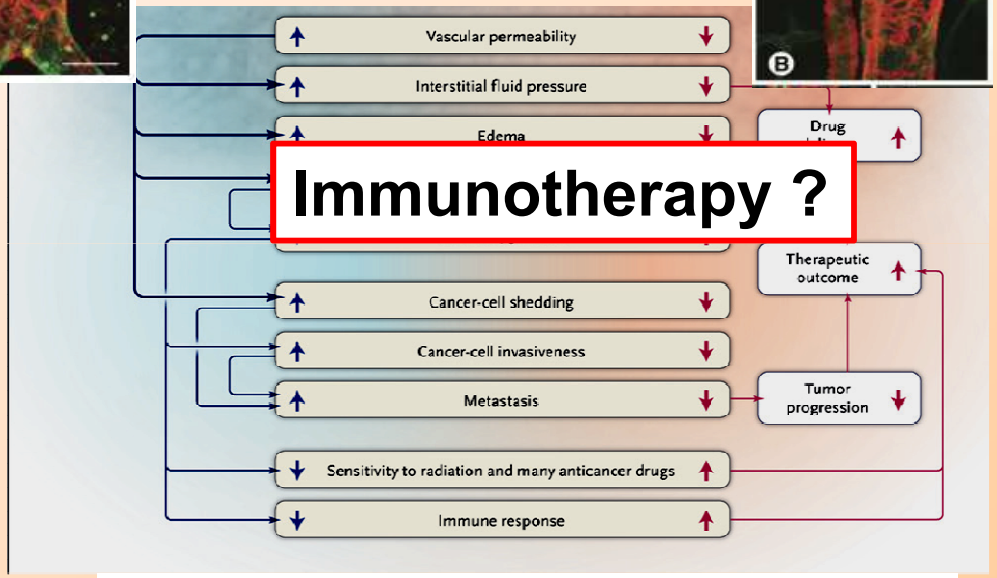


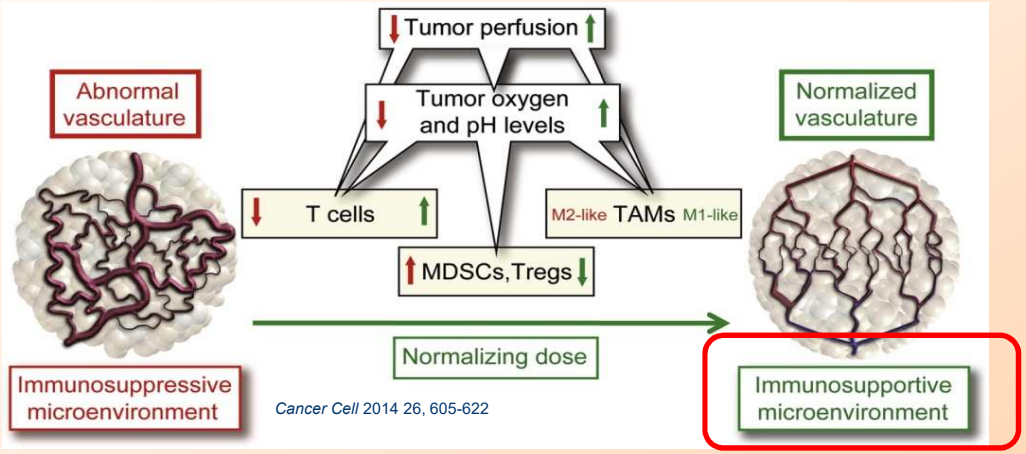
Carmeliet et al. Current Opinion in Genetics & Development 2011, 21:73–79



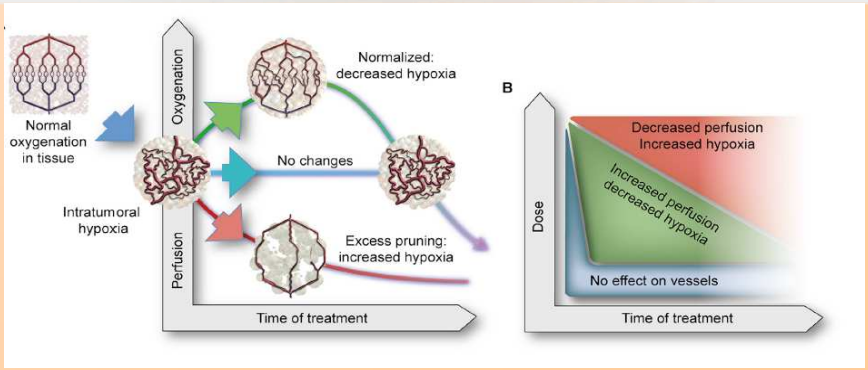
et al.Physiol Rev 91: 1071–1121, 201







Cancer Cell 2014 26, 605-622

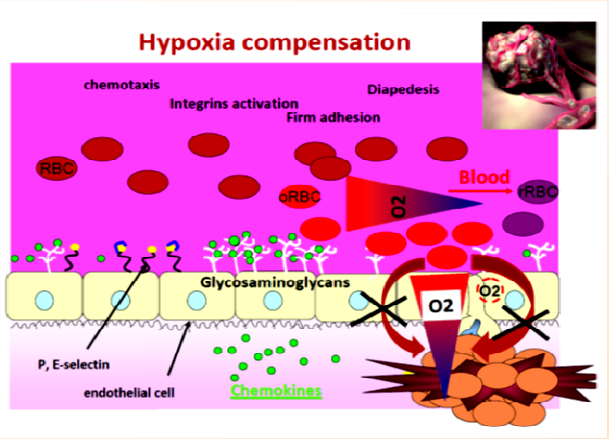


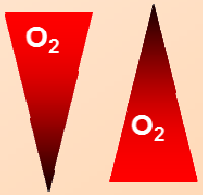
Angiogenesis normalization by hypoxia compensation

How does repair of the endothelial damage change tumor microenvironment ?

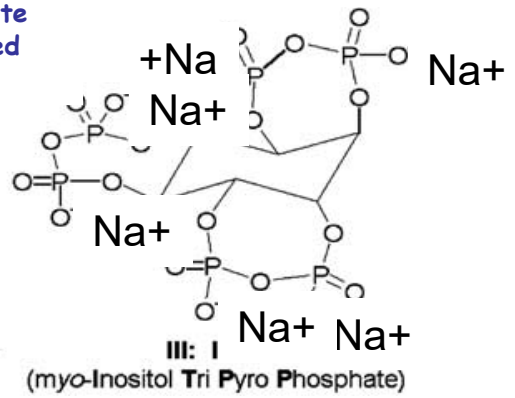
✓ **Strategy directed to hemoglobin properties**

Hemoglobin carries oxygen



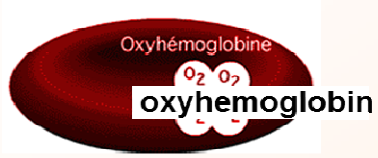


Cyclic pyrophosphate able to be delivered into RBCs

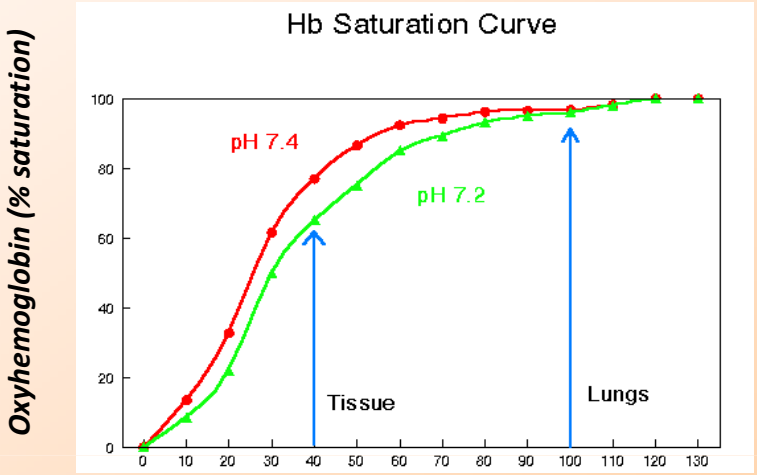


Can ITPP increase oxygen delivery ?

Hemoglobin carries oxygen

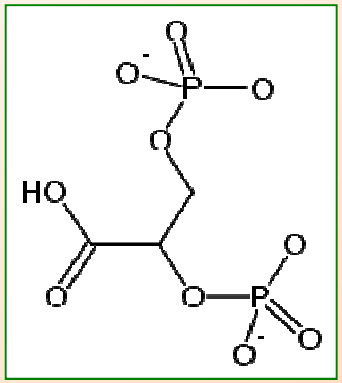


Oxygen delivery can be increased if oxygen affinity for hemoglobin is decreased

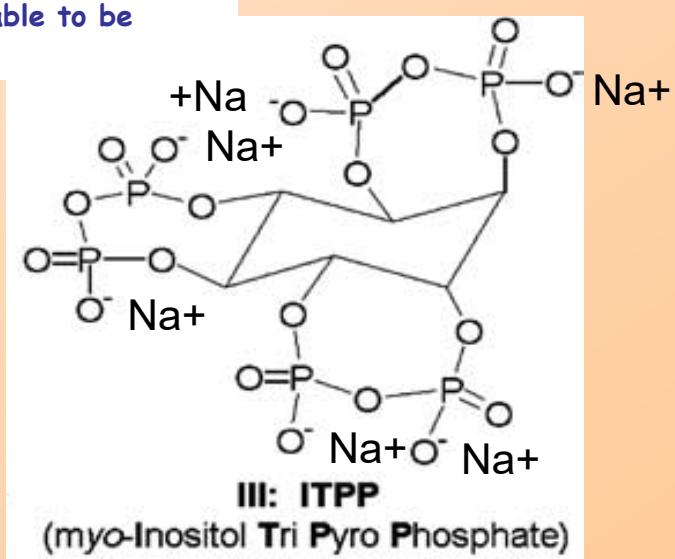


Decrease oxygen affinity for hemoglobin:

- pH decrease
- 2,3-diphosphoglycerate (DPG)



Cyclic pyrophosphate able to be delivered into RBCs



Duarte CD et al, Chembiochem. 2010)

Hypoxia compensating strategies are normalizing vessels:

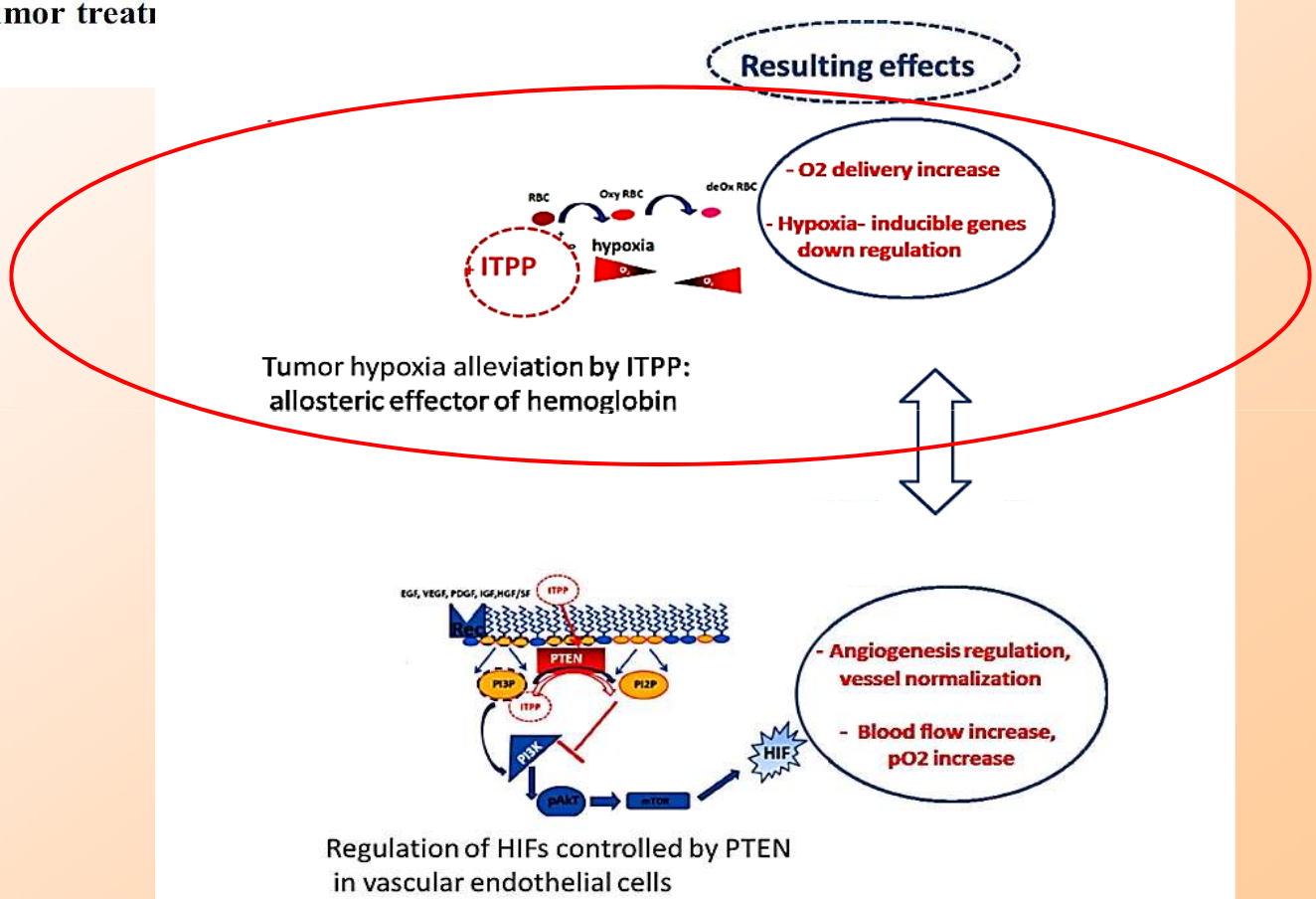
J Mol Med
DOI 10.1007/s00109-013-0992-6

JMolMed

ORIGINAL ARTICLE

Stable tumor vessel normalization with pO_2 increase and endothelial PTEN activation by inositol trispyrophosphate brings novel tumor treati

Claudine Kieda · Bouchra El Hafny-Rahbi ·

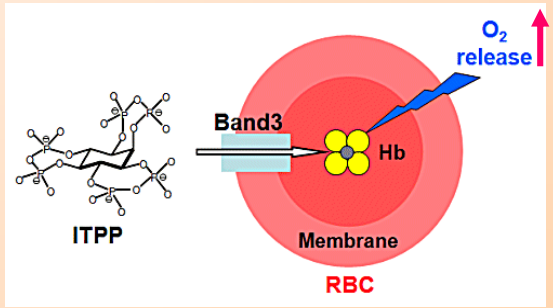


myo-Inositol Trispyrophosphate (ITPP): A novel allosteric effector of hemoglobin with high permeation selectivity across the red blood cell plasma membrane:

ITPP uptake is mediated by Band 3 protein *(Duarte CD et al, Chembiochem. 2010)*

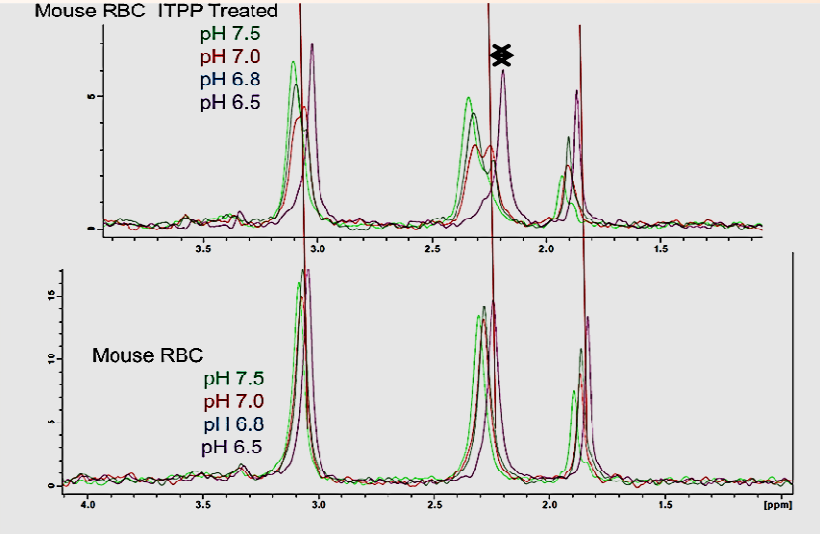
Band 3: anion exchanger (AE1) mediates oxygen-regulated metabolic transitions in RBC

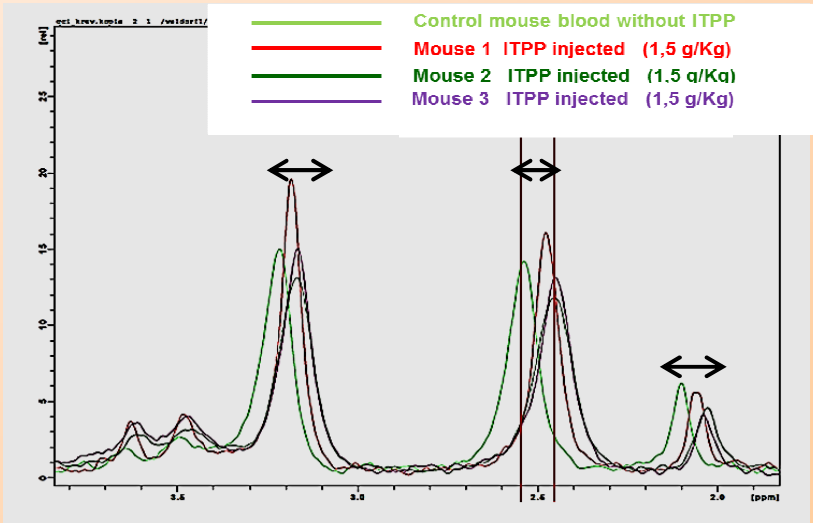
Elevates glycolytic fluxes in deoxygenated erythrocytes by displacing the glycolytic enzymes from their inibitory site on Band 3 *(Lewis et al, PNAS 2009)*



Lowers the pH in RBC ?

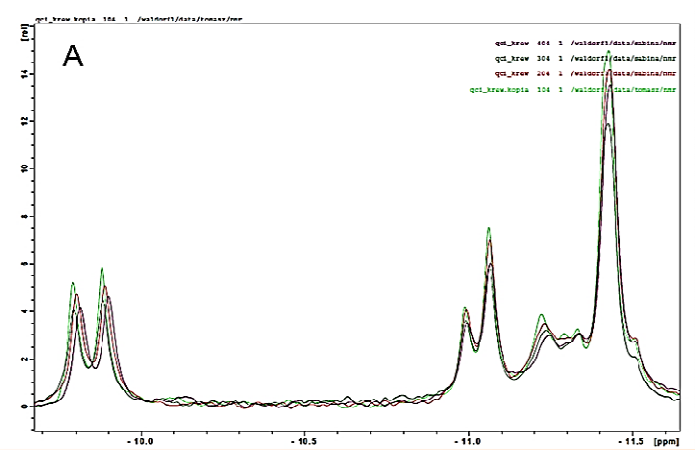
31P NMR analysis 2,3 DPG region chemical shifts acidification



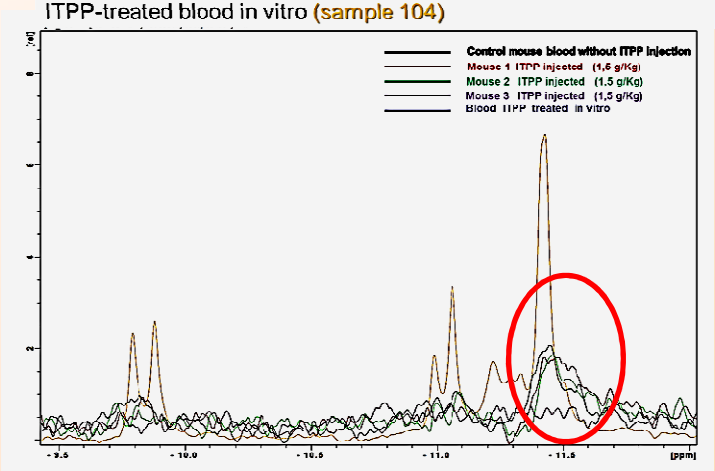


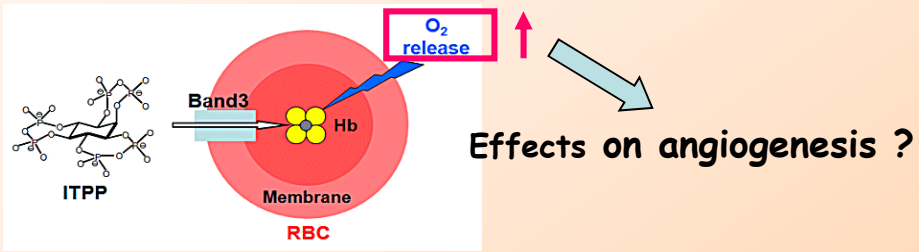
NMR ITPP detection in vivo

Analysis in the ITPP region of resonance (-9 to -16ppm)
Analysis of mouse blood (104 to 404) pH from 7.5 to 6.5: ITPP added *in vitro* 30mg/mL of blood:

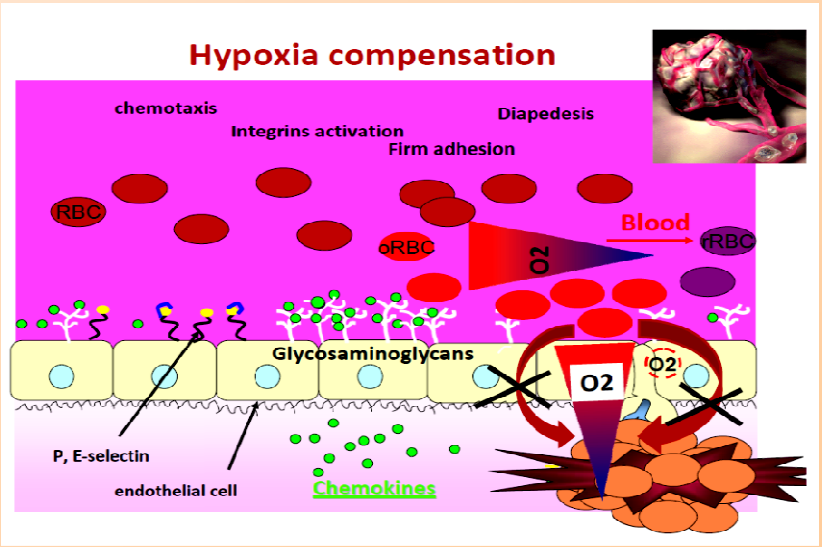


Analysis in the ITPP region of resonance (-9 to -16ppm)
Analysis of mouse blood of mice treated by ITPP in vivo1,5 G/Kg
ITPP-treated blood in vitro (sample 104)





Effects on angiogenesis ?



New strategies for imaging angiogenesis structure and functions

Evolution of angiogenesis: an important target for novel anticancer therapeutics…….. new challenges for *in vivo imaging*

Optical methods : bioluminescence , fluorescence

Whole animal imaging : functions MRI, MRA, DCEMRI, BOLD MRI

Multi modal imaging

In clinical practice:
•angiogenesis imaging in diagnosis, staging and response monitoring
•assessment of angiogenic process /structural / functional and molecular levels, before, during and after antiangiogenic therapy.

Photoacoustic Oxygen Saturation

Tumor oxygen saturation and hemoglobin concentration can be quantified. Overall, the tumor has very high oxygen saturation. ROIs were drawn for the whole tumor (green) and for a more hypoxic center region (blue).

Microvascularization in the F98 rat model of glioma

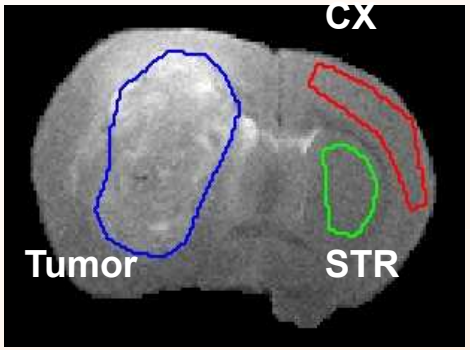
Grenoble Neurosciences Institute– U836

Blood Oxygen Level Dependent BOLD MRI assessment

Tumor Model and Treatment: Tumor F98-Fischer Rat

Treatment start: **T0**, 23 days after tumor inoculation: **D23**

MRI parameters:



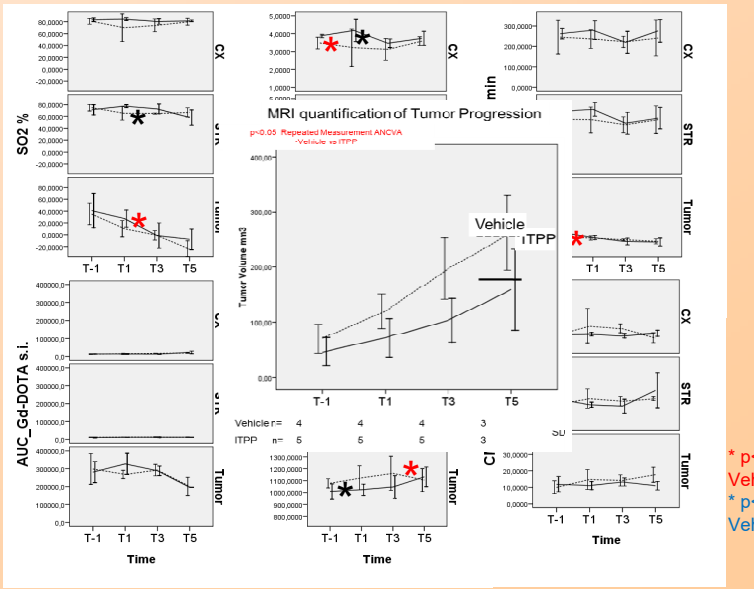
On 3 Regions of Interest (ROI): Cortex (CX), Striatum (STR) and Tumor

- anatomy with T_2W map
- apparent diffusion constant (ADC) ~ oedema, necrosis
- cerebral blood volume (CBV)
- cerebral blood flow (CBF)
- integrity of the vascular wall ("permeability"):
  area under curve after Gd-DOTA injection ($AUC_{Gd\text{-}DOTA}$)
- tissue oxygen saturation (SO_2) ~ hypoxia
- cerebral metabolic rate of oxygen ($CMRO_2$) ~ oxygen consumption

MRI follow-up: D22T-1, D24T1, D26T3, D28T5

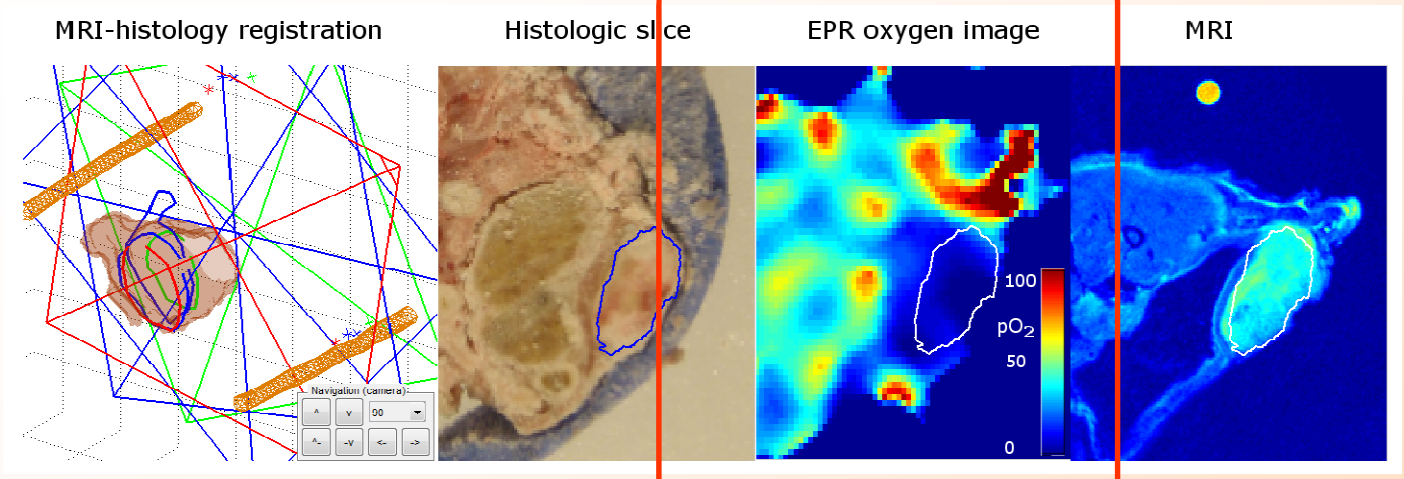
Value intervals for each MRI map

ADC	0/5000 µm²/sec
CBV	0/20 %
CBF	0/400 mL/100g/min
SO2	-150/100 %
$AUC_{Gd\text{-}DOTA}$	0/1 250 000 s.i.
CMRO2	0/100 mL/100g/min
R2prim	0/30 sec^{-1}



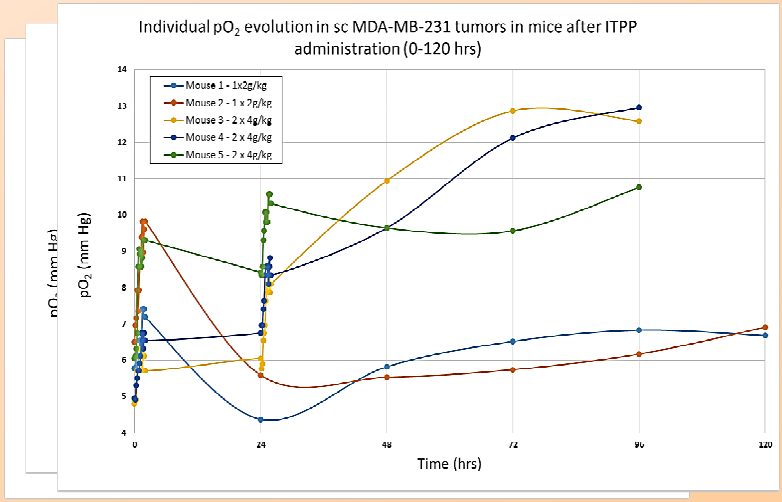
* p<0.05 ANOVA, Vehicle vs ITPP
* p<0.1 ANOVA , Vehicle vs ITPP

ESE and MRI Image Registration



Real PO2 value
Non invasive
Trityl iv perfusion

Model	Baseline value	During carbogen breathing	Improvement (%)
9L-Glioma	8.8 ± 1.1	16.6 ± 1.9	89%
Rhabdomyosarcomas	5.3 ± 0.7	8.7 ± 0.6	64%
SiHa - squamous cell carcinoma (cervix)	4.9 ± 1.1	16.0 ± 2.3	227%
MDA-MB-231 Adenocarcinoma (mammary)	3.8 ± 2.0	9.9 ± 0.9	161%
NT2 - Mouse mammary tumor	4.8 ± 3.0	7.9 ± 0.8	65%
FSall - mouse fibrosarcoma	5.3 ± 0.3	8.0 ± 0.3	51%



Stabilization of the elevated pO2 corresponds to stable vessel normalization:
effects on tumor evolution

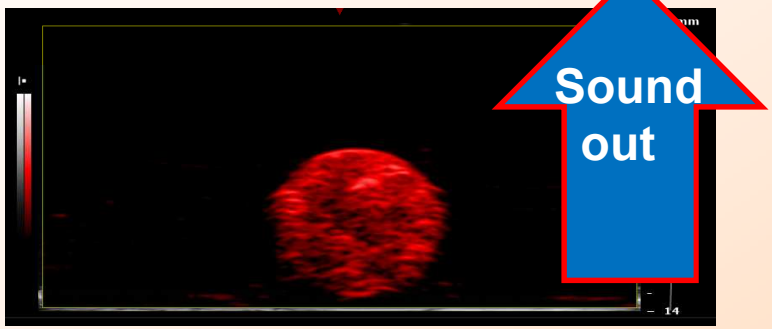
The Photoacoustic Effect

Visual Sonics

Ultrasound

Optical

4) Detection of ultrasound and creation of image



Sound out

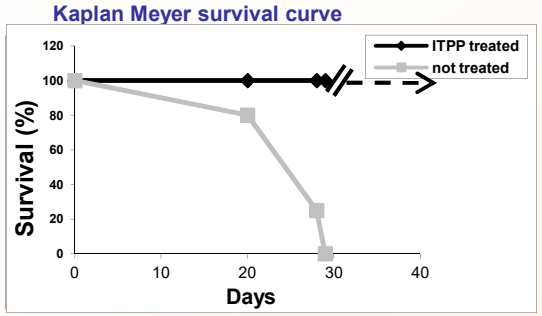
Light in

1) Nanosecond laser pulse illumination

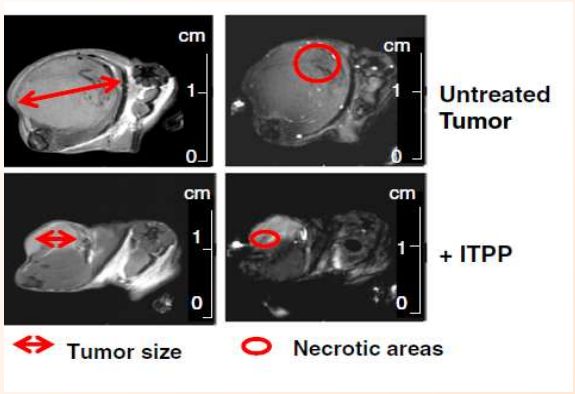
3) Emitted pressure (sound) wave

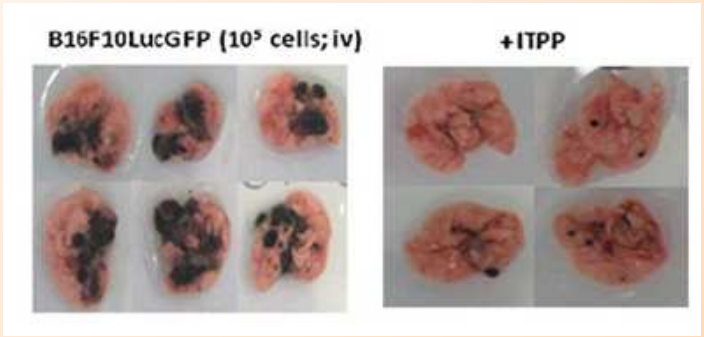
2) Optical absorption, heating and thermoelastic expansion

Hypoxia compensation affects tumor cell resistance

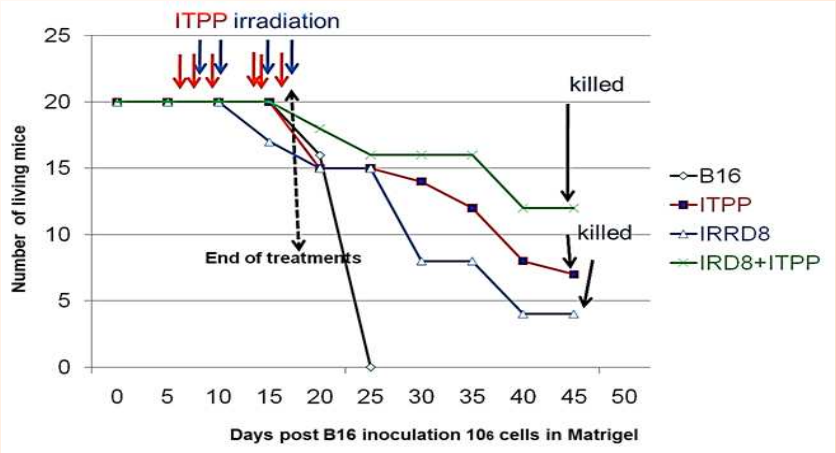


D0: B16 Luc cells injection
D 7,8 14, 15, 21, 22, 28, 29: ITPP injection

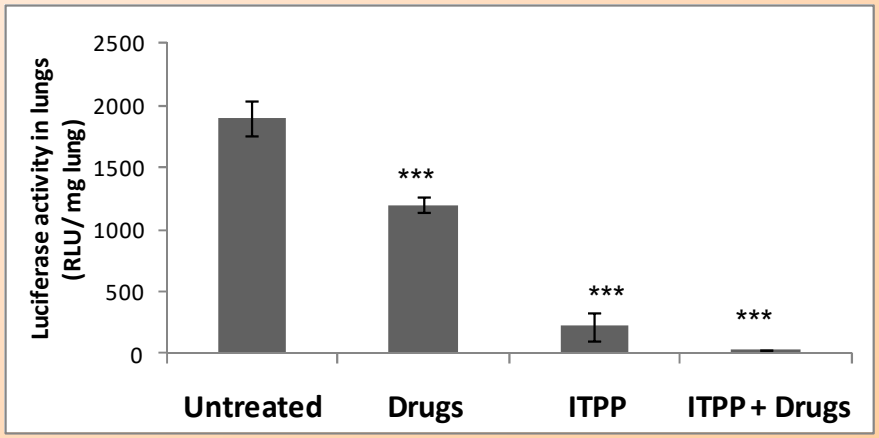




Irradiation



Synergy on Drug effect



Molecular mechanism:

1) pO2 increase

2) endothelial PTEN activation

stable vessel normalization

Hypoxia compensating strategies are normalizing vessels:

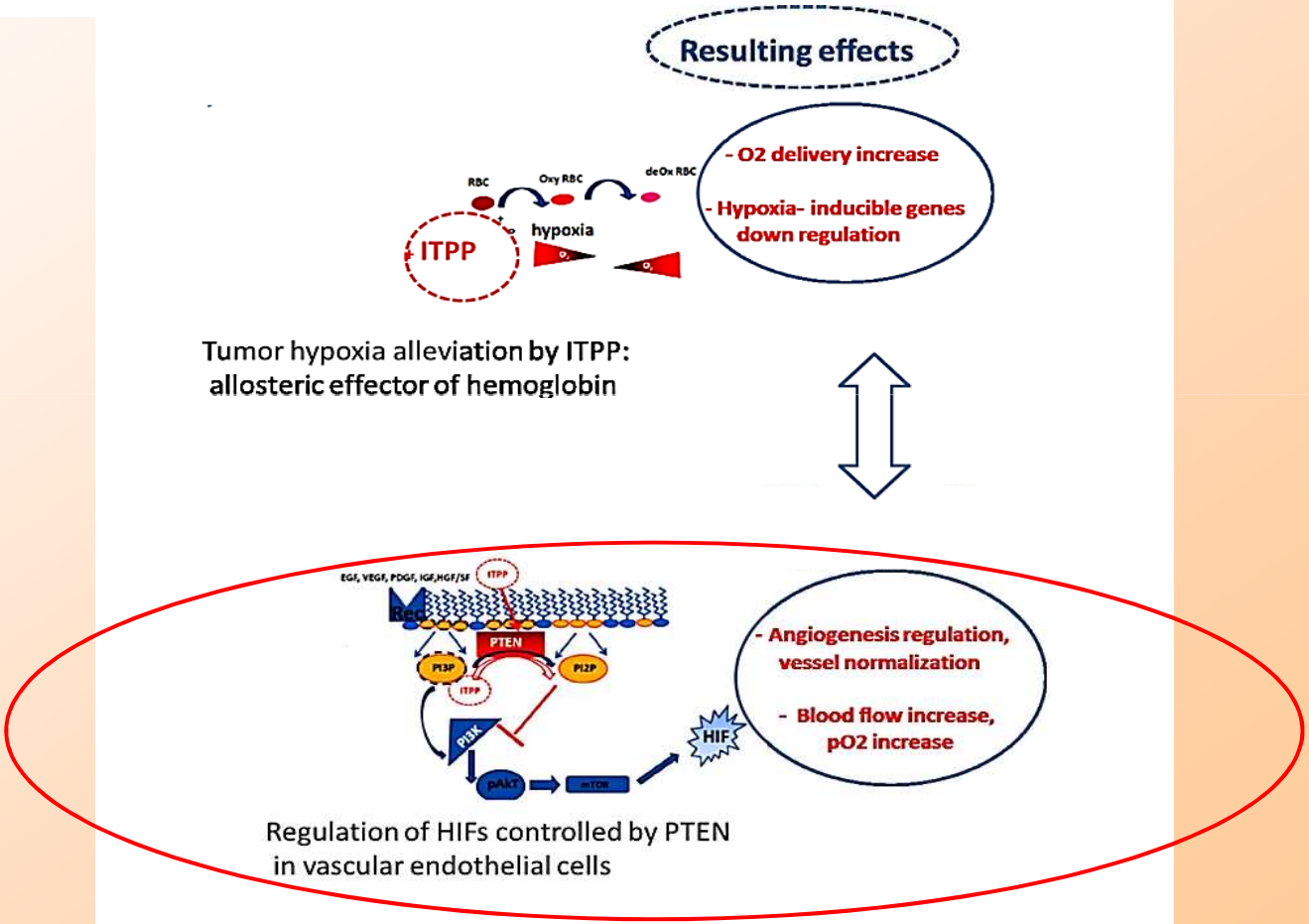
J Mol Med
DOI 10.1007/s00109-013-0992-6

JMolMed

ORIGINAL ARTICLE

Stable tumor vessel normalization with pO_2 increase and endothelial PTEN activation by inositol trispyrophosphate brings novel tumor treatment

Claudine Kieda · Bouchra El Hafny-Rahbi ·



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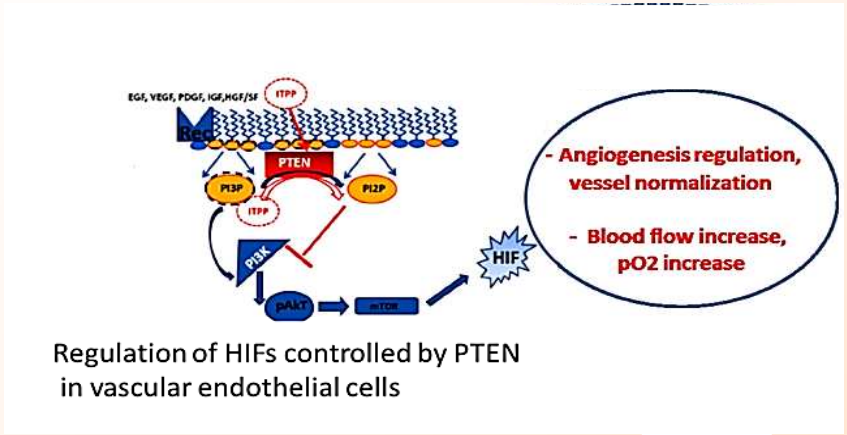
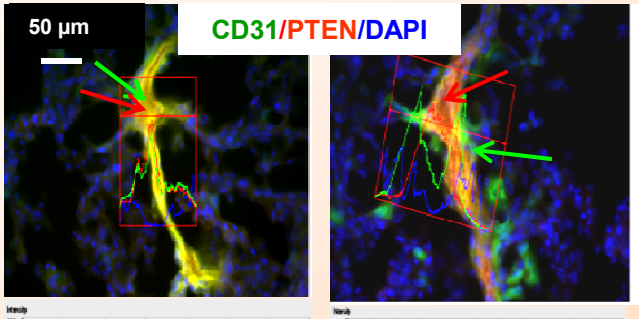
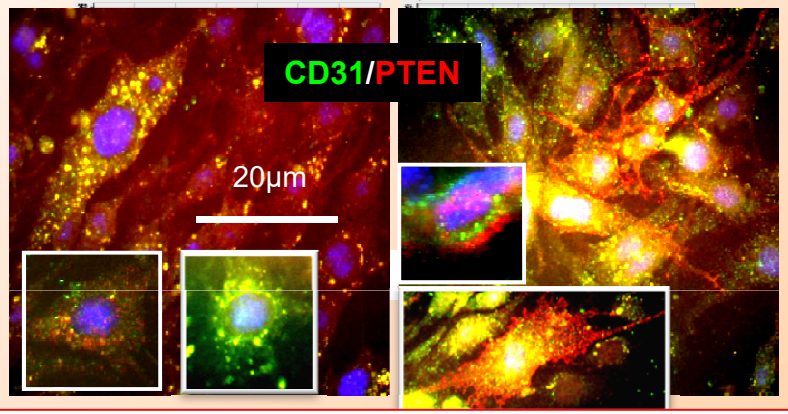


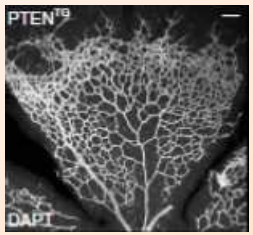
Regulation of HIFs controlled by PTEN in vascular endothelial cells

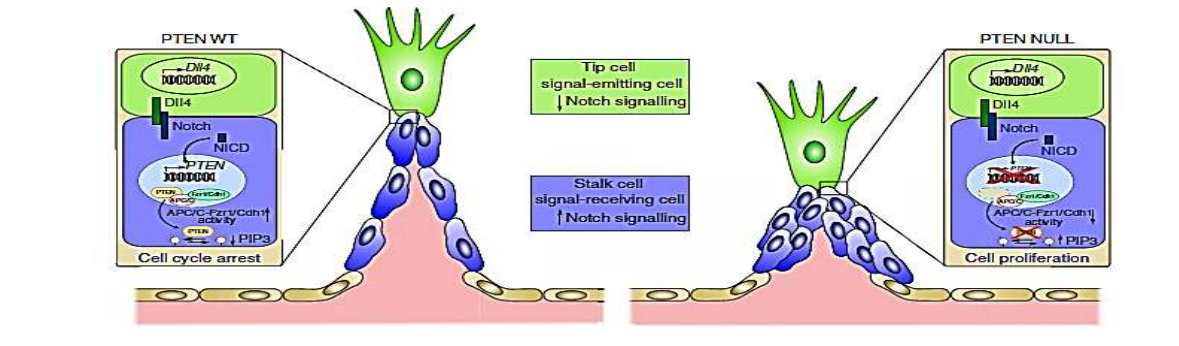


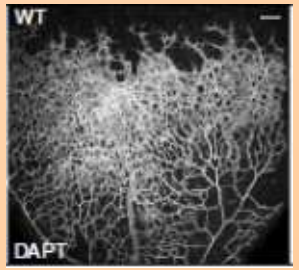


Is ITPP a ligand for PTEN ?

Effect on angiogenesis in hypoxia: Notch4/PTEN mediating stalk cell arrest







The Role of PTEN in TumorAngiogenesis

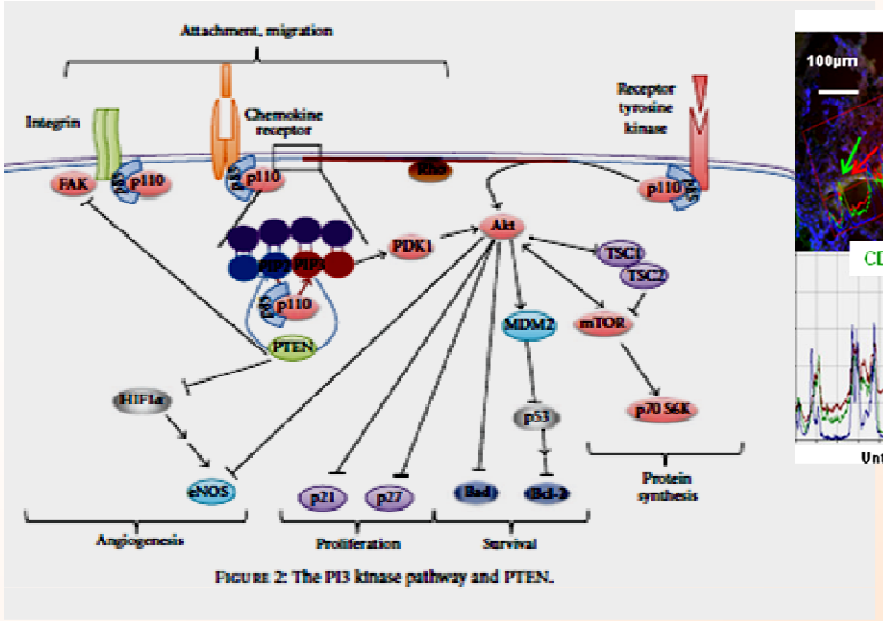
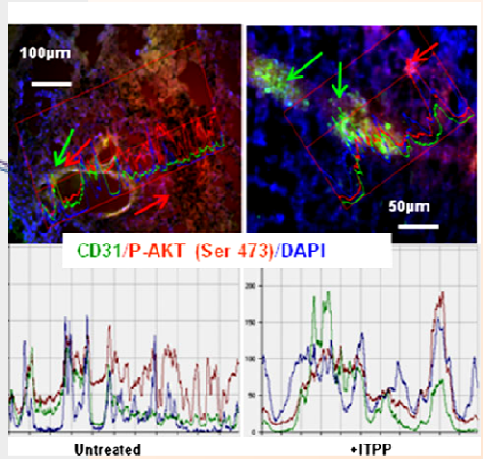


FIGURE 2: The PI3 kinase pathway and PTEN.



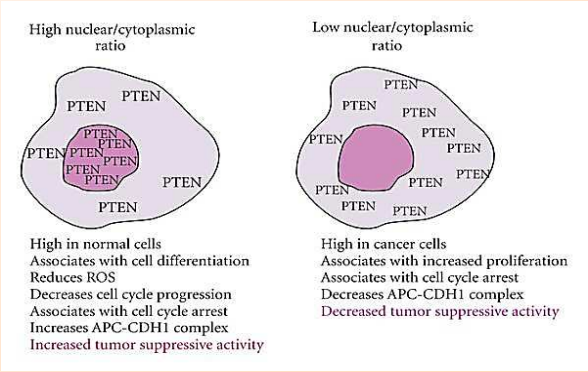
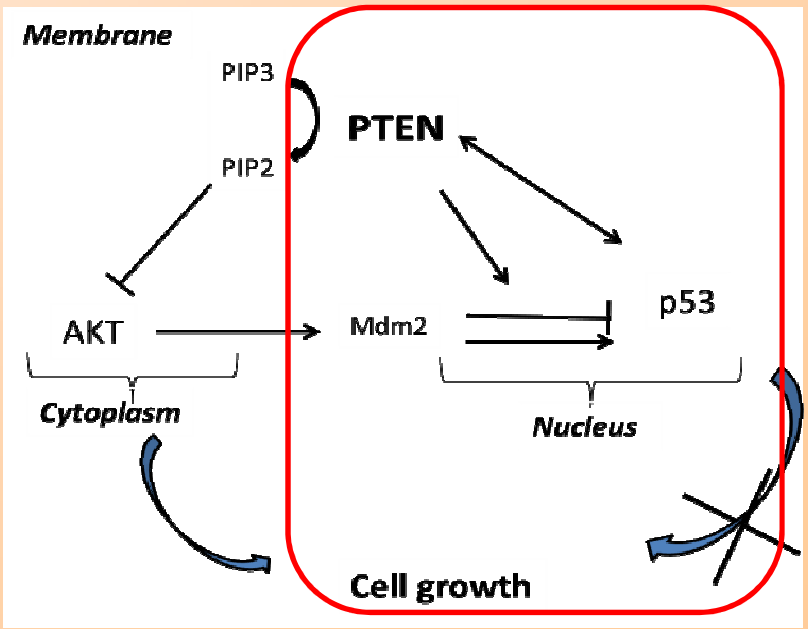


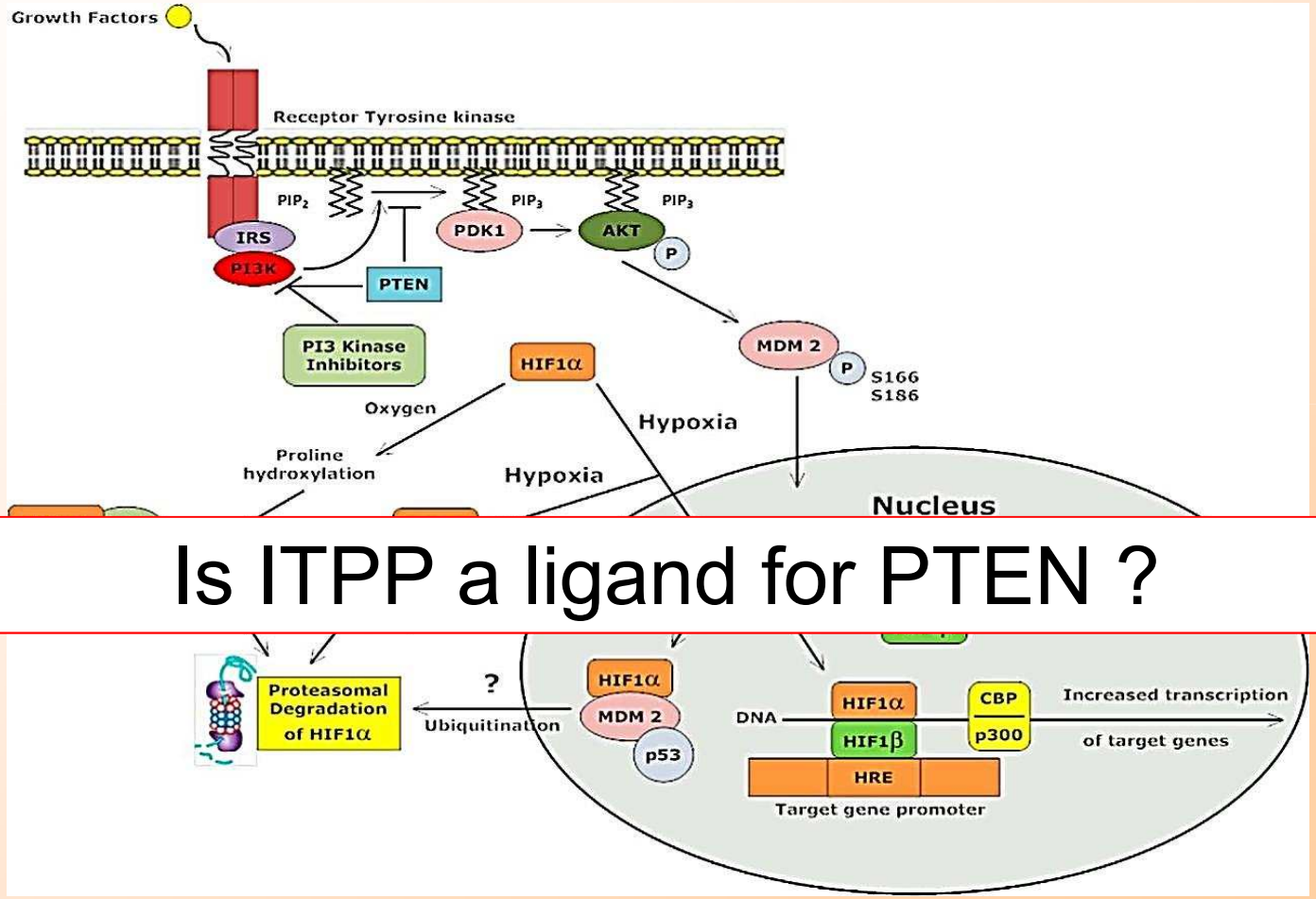
TABLE 2: Clinical trials having shown an impact of the PTEN status on the response to cancer treatment.

Type of cancer	Metastatic form	Treatments
Colorectal		Cetuximab, panitumab
	×	Cetuximab (+irinotecan)
Breast		Trastuzumab, lapatinib
		Trastuzumab
	×	Endocrine therapy
Glioblastoma		Gefitinib, erlotinib
		Erlotinib + temozolomid
Gastric		Streptozotocin, doxorubicin, 5-fluorouracil, etoposide/cisplatinum
	×	Streptozotocin, doxorubicin
Lung		Gefitinib, erlotinib
	×	Gefitinib, erlotinib
Pancreas		Gemcitabine
Esophageal		5-fluoropyrimidine, taxane, platinum, PI3K pathway inhibitor



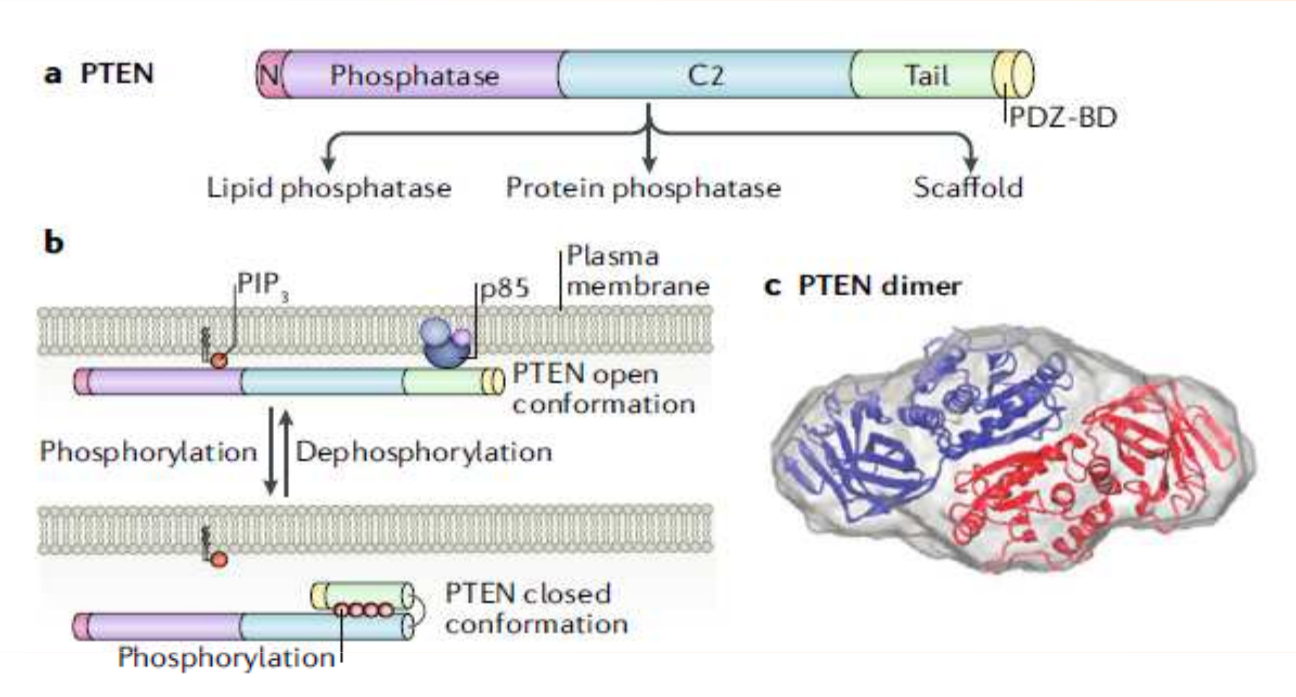
Important to stabilize PTEN activation

Mechanism of PTEN-PI3K-AKT-MDM2 signaling axis control over HIF1α degradation in cytoplasm under hypoxic conditions via the MDM2 E3 ligase and 26 S proteasome

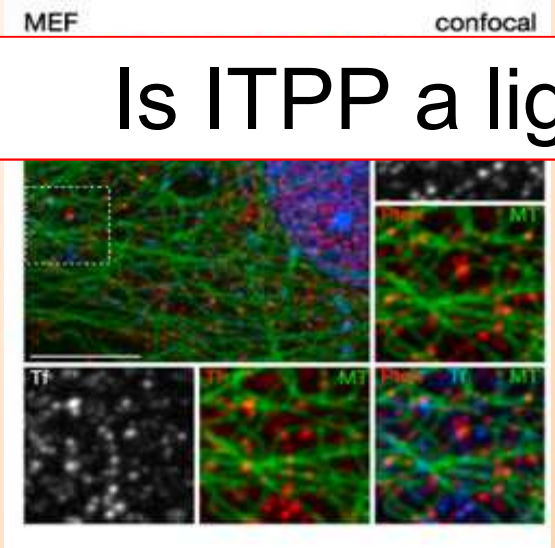


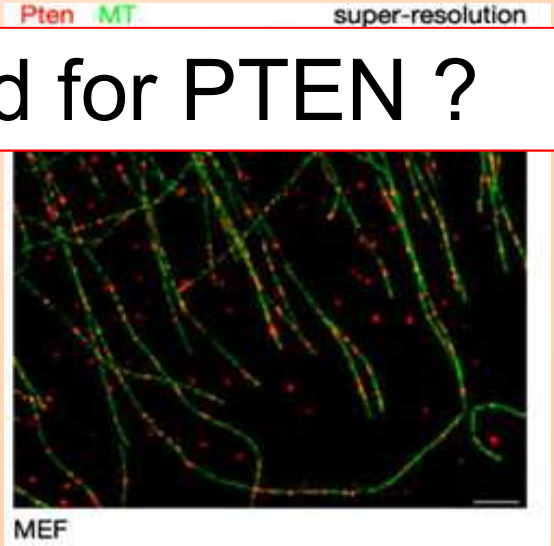
Is ITPP a ligand for PTEN ?

Shweta Joshi et al. J. Biol. Chem. 2014;289:22785-22797



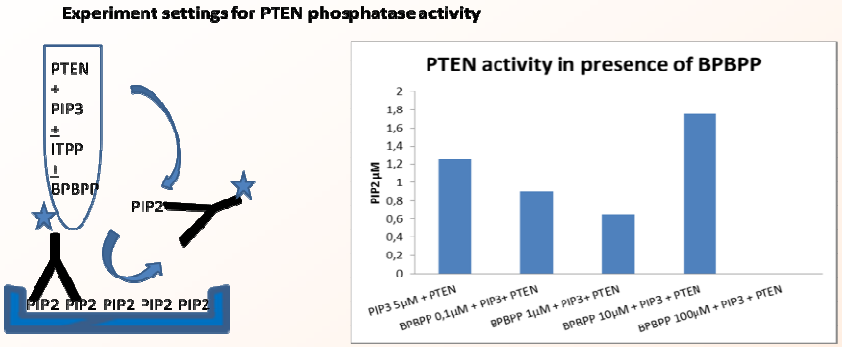
Is ITPP a ligand for PTEN ?



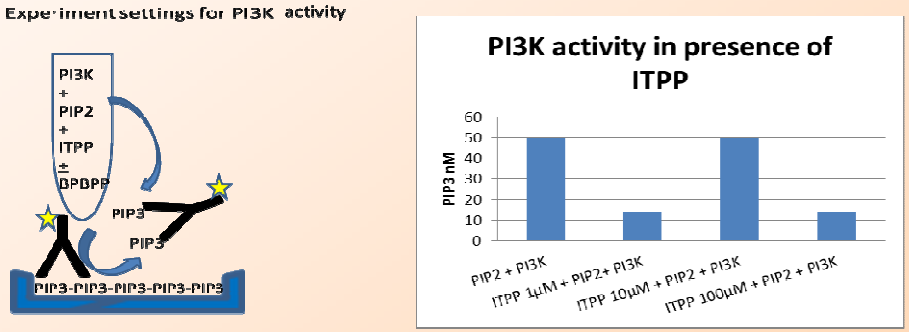


Lee et al. Nat. Rev. Mol. Cell Biol. 2018

Activity is measured as the inhibitory effect of antibody binding to PIP2

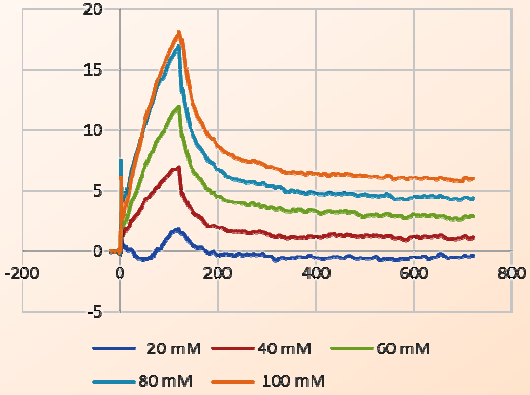


Activity is measured as the inhibitory effect of antibody binding to PIP3

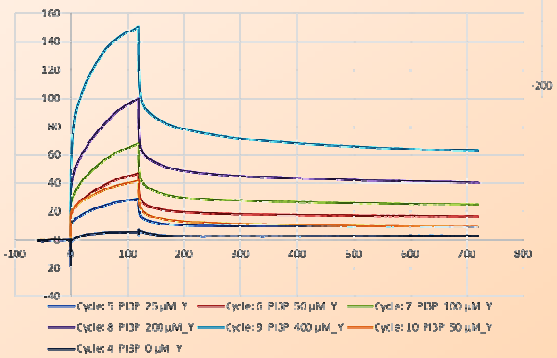


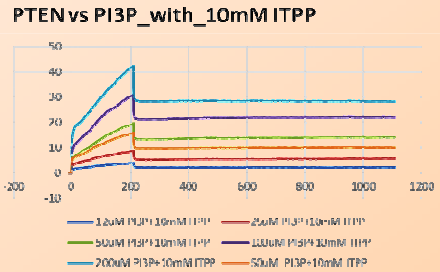
Surface plasmon resonance analysis of ITPP/PTEN kinetics

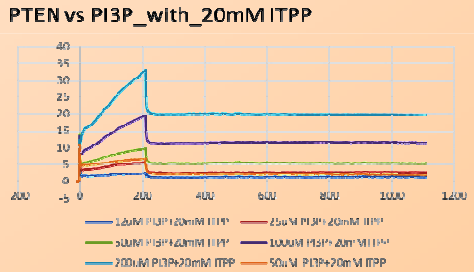
PTEN vs ITPP

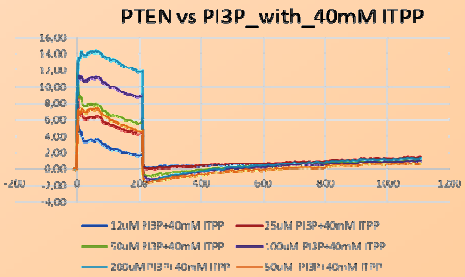


PI3P

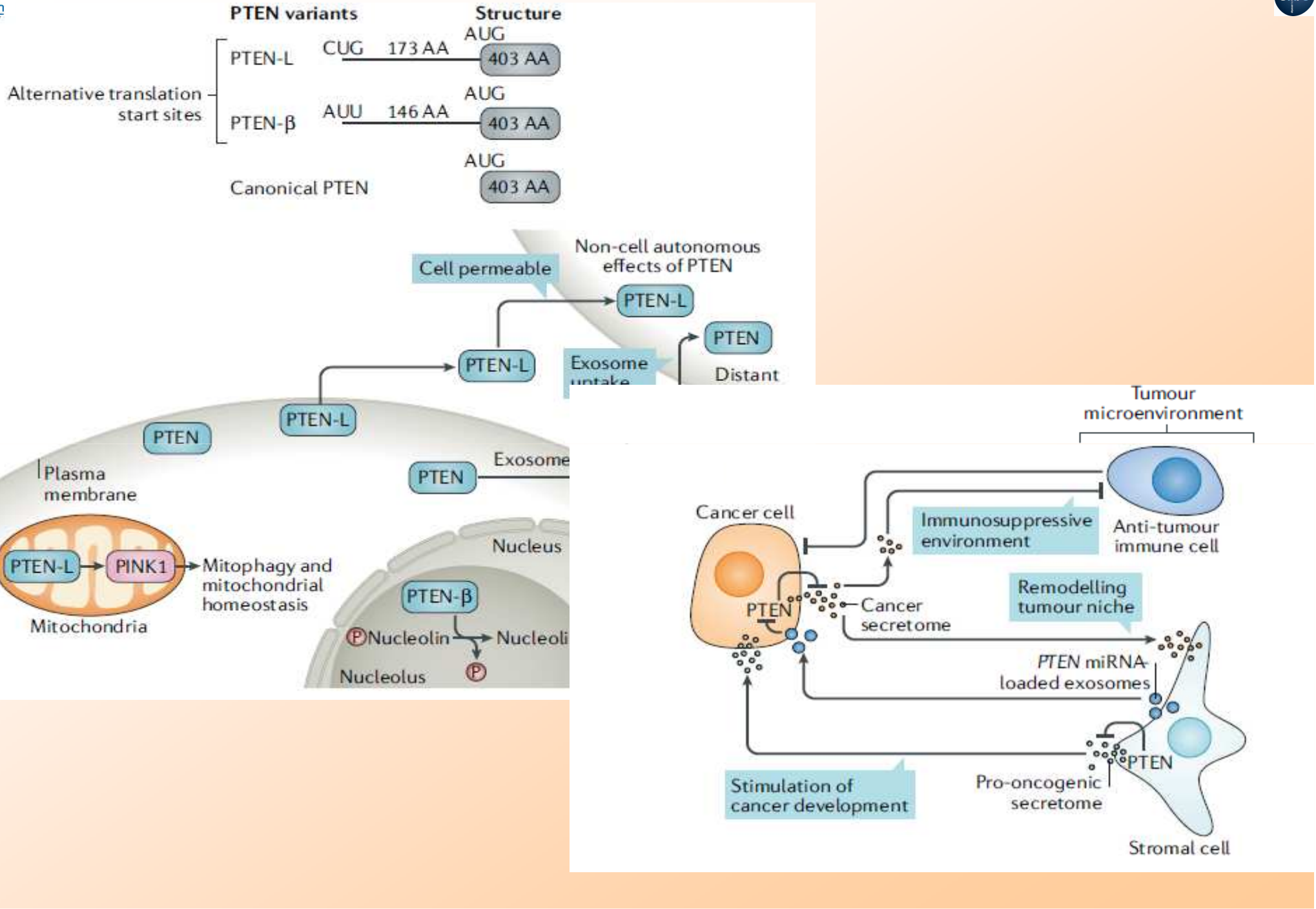






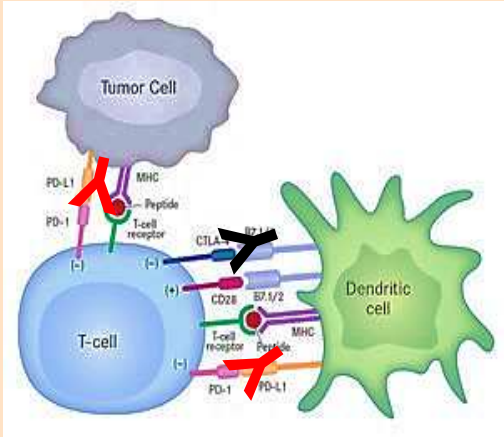


ITPP is a ligand for PTEN, allosteric phosphatase activator /inhibitor for PI3K


PTEN variants
Structure
Alternative translation start sites
PTEN-L
CUG
173 AA
AUG
403 AA
PTEN-β
AUU
146 AA
AUG
403 AA
Canonical PTEN
AUG
403 AA
Non-cell autonomous effects of PTEN
Cell permeable
PTEN-L
PTEN
Exosome uptake
Distant
PTEN-L
PTEN-L
PTEN
Plasma membrane
Exosome
PTEN
PTEN-L
PINK1
Mitophagy and mitochondrial homeostasis
Mitochondria
Nucleus
PTEN-β
Nucleolin
Nucleoli
Nucleolus
Tumour microenvironment
Cancer cell
Immunosuppressive environment
Anti-tumour immune cell
PTEN
Cancer secretome
Remodelling tumour niche
PTEN miRNA-loaded exosomes
PTEN
Pro-oncogenic secretome
Stimulation of cancer development
Stromal cell

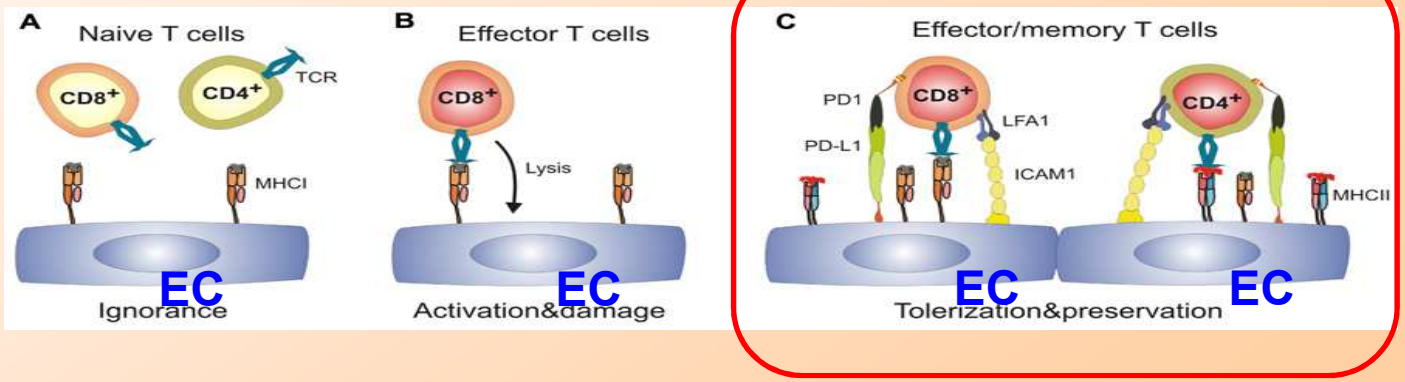
Hypoxia compensation and immune response

Endothelial cells induce tolerance via PD-L1/2 (Tewalt et al. Blood 2012) **in HYPOXIA**
PD1 Ligands expression is controlled by PTEN



Y Anti PD1, anti PD-L1/L2

Y Anti CTLA4

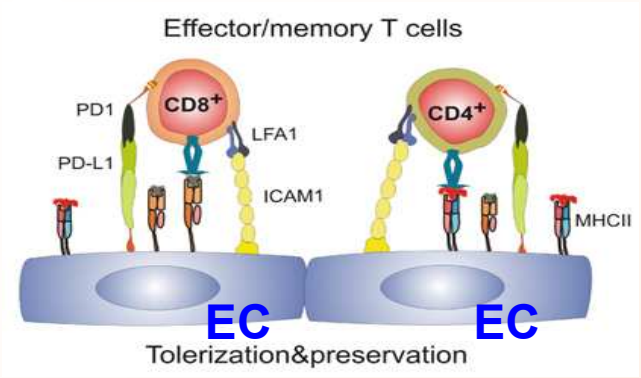


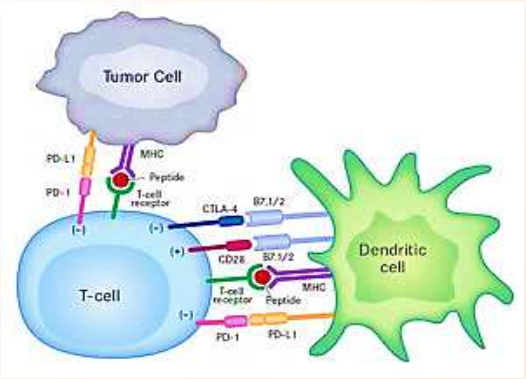
PD1/PDL1/2 is hypoxia mediated

PD-L1 is a novel direct target of HIF-1a, and its blockade under hypoxia enhanced MDSC-mediated T cell activation Noman *et al.* JEM 2014

O2 increase: in ECs PTEN is activated and express less PD1 Ligands

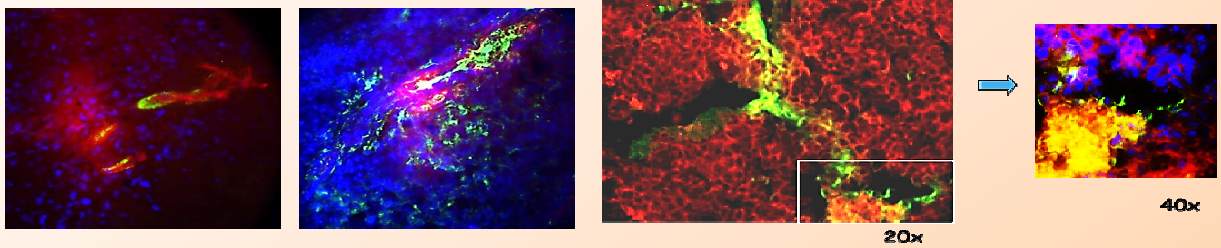
Hypoxia compensation and immune response



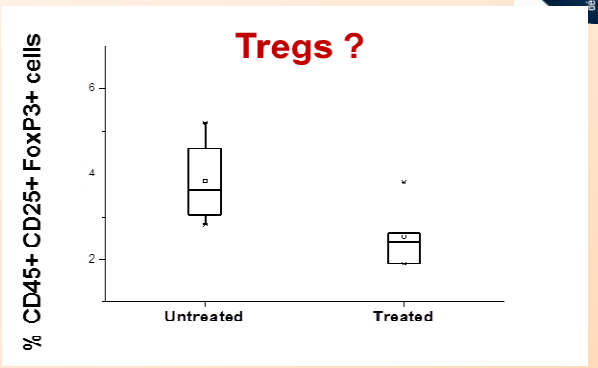


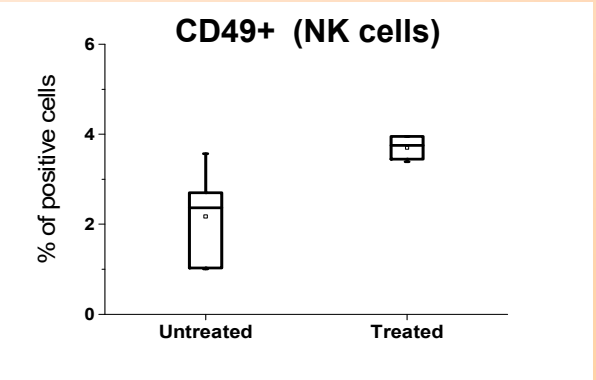
Endothelial cells induce tolerance via PD-L1/2 in HYPOXIA

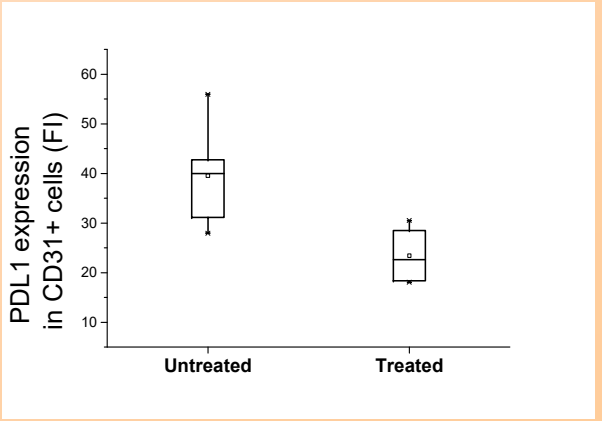
NKs in tumors B16F10



Control treated



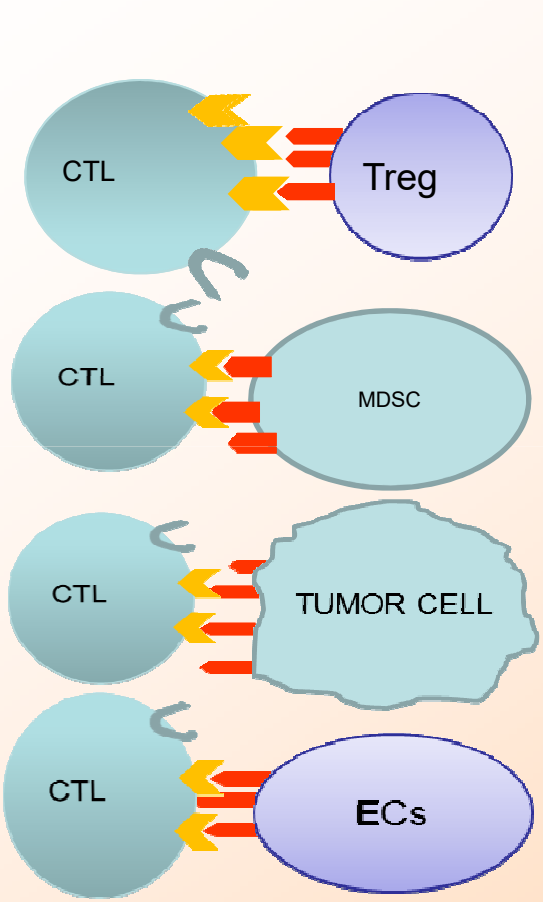


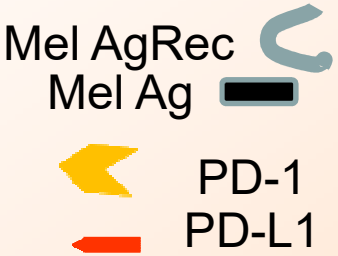


O2 increased ECs express less PD1 Ligands

PD-1/PD-L1 pathway is hypoxia dependent

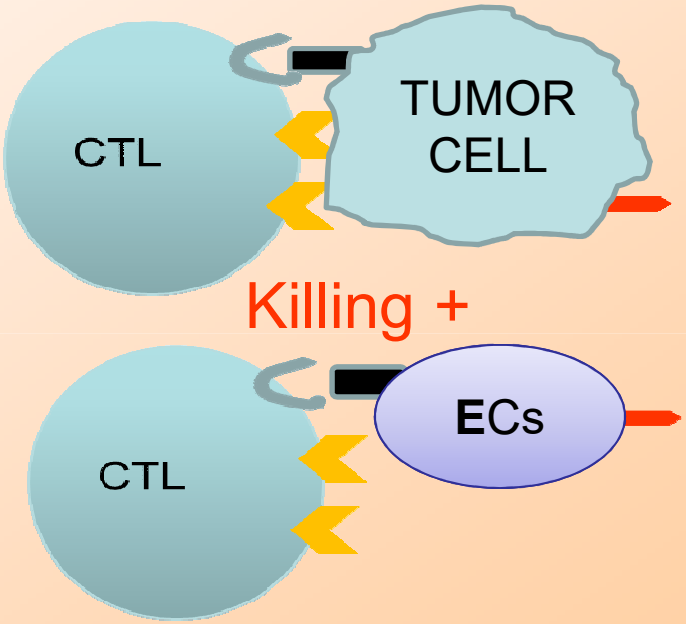
Hypoxia destroys the CTL-specific killing





Hypoxia induced PD-L1/2 expression
Increases suppression by Tumor cells, Tregs, MDSC and ECs

Treatment by :
hypoxia compensation



Killing restoration of tumor cells
Tumor endothelial cells
PD-L1 expression is modulated in Ecs and tumor cells as melanoma

Other immune checkpoints ?

Opportunities for pharmacological PTEN reactivation after partial or complete loss of *PTEN* expression

Applicable tumour type	Therapeutic intervention
Tumours with complete loss of *PTEN*	• Administer PTEN-Long protein • Administer PTEN nanoparticles
Tumours with monoallelic *PTEN* deletion or intact *PTEN*	• Increase PTEN dimerization • Use drugs to increase the activity of PTEN transactivators • Inhibit *PTEN*-targeting microRNA • Derepress epigenetic silencing or histone deacetylation • Target E3 ligase to stabilize PTEN protein
Tumours with monoallelic *PTEN* mutation	• Administer PTEN-Long protein • Administer PTEN nanoparticles • Block the dimerization between mutant and wild-type PTEN • Edit the *PTEN* gene to correct mutations or engineer into enhanced *PTEN* variants

Conclusion

The angiogenesis normalization-induced activation of endothelial PTEN opens the perspective of compensating the tumor suppressor default in tumors

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Military Institute of Medicine