



Recent drugs: Trabectedin

Maurizio D'Incalci, MD

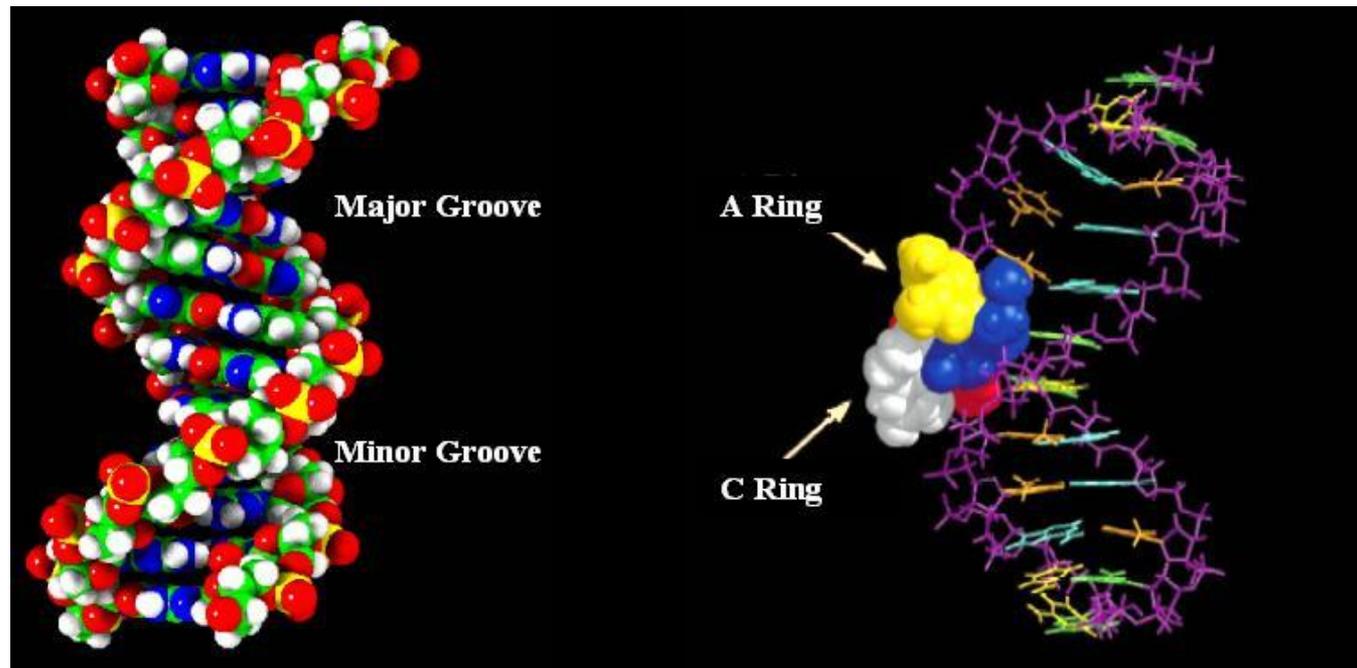
IRCCS – Istituto di Ricerche
Farmacologiche Mario Negri

Disclosure slide

- Receipt of grants / research supports:
Pharma Mar
- Receipt of honoraria or consultation fees:
Pharma Mar

Trabectedin: mechanism of action

- Trabectedin binds to the minor groove and bends DNA towards the major groove



Clinical Development of Trabectedin in STS

Study	Reference	Regime	Types of tumours	n	
Single Arm Study	Le Cesne 2005 ¹	• 1.5 mg/m ² 24-hr q3wk	STS, liposarcoma, leiomyosarcoma, synovial sarcoma, malignant fibrous histiocytoma, neurosarcoma, others	104	} Single-arm Trials (Pooled data)
Single Arm Study	Yovine 2004 ²	• 1.5 mg/m ² 24-hr q3wk	STS, leiomyosarcoma, liposarcoma, GIST, synovial sarcoma, malignant fibrous histiocytoma, fibrosarcoma, others	54	
Single Arm Study	García Carbonero 2004 ³	• 1.5 mg/m ² 24-hr q3wk	STS, liposarcoma, leiomyosarcoma, malignant Schwannoma	36	
Randomised study	Demetri 2009 ⁴	• 1.5 mg/m ² 24-hr q3wk • 0.58 mg/m ² 3-hr qwk for 3 out weeks	Liposarcoma and leiomyosarcoma	270	} Randomised Trial

1. Le Cesne A, et al. J Clin Oncol. 2005;23(3):576-84.

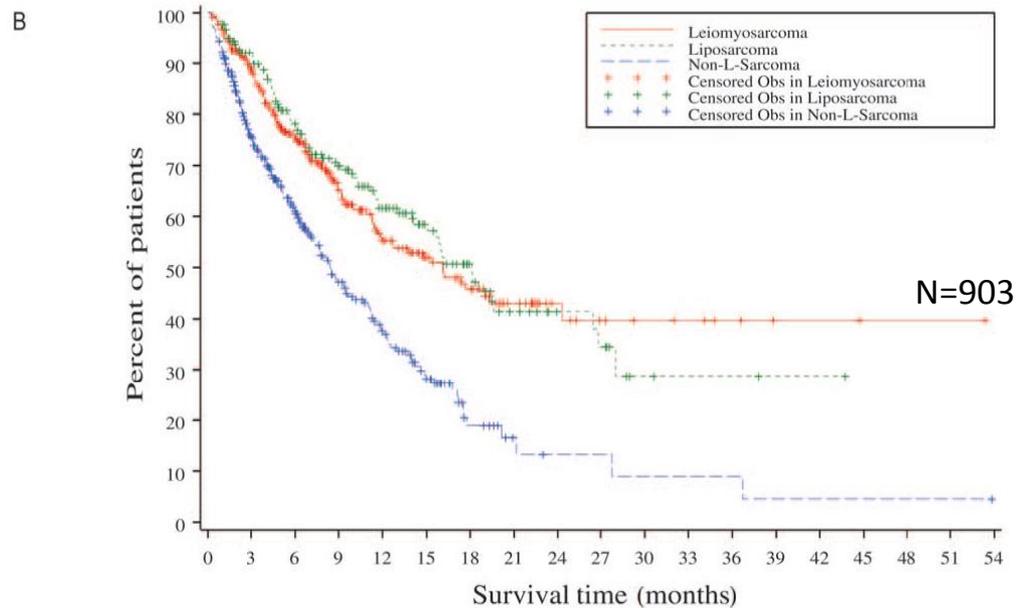
2. Yovine A, et al. J Clin Oncol. 2004;22(5):890-9.

3. Garcia-Carbonero R, et al. J Clin Oncol. 2004;22(8):1480-90.

4. Demetri GD, et al. J Clin Oncol. 2009;27(25):4188-96.

Real Life Data Trabectedin Worldwide Expanded Access

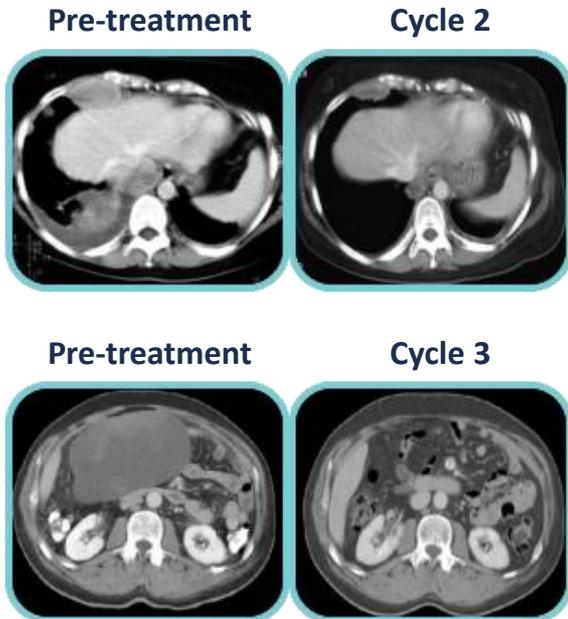
Median Survival:	11.9 months
Non-L Sarcoma Survival	8.4 months
LMS Survival	16.2 months
LPS Survival	18.1 months



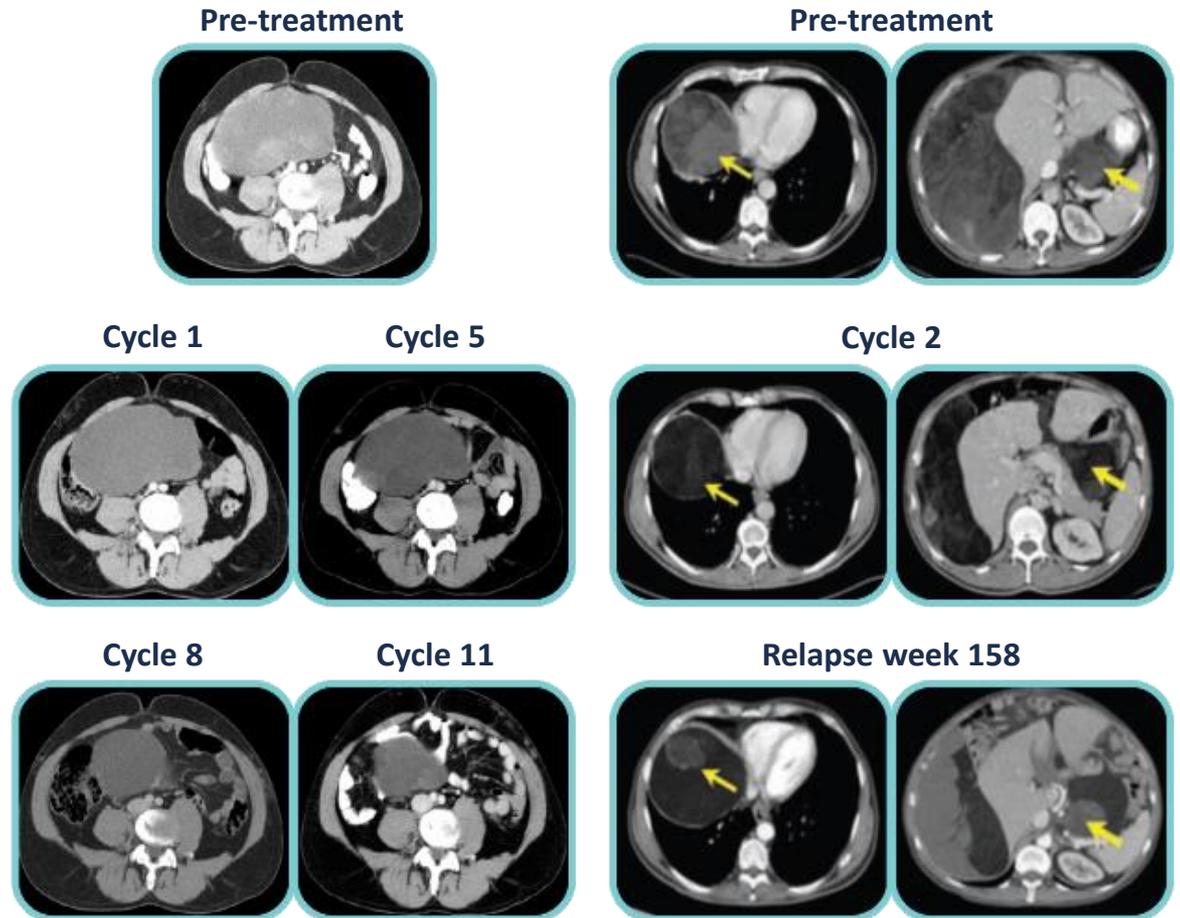
Clinical patterns of response to Trabectedin

Atypical pattern of response

Classical pattern



Adapted from Grosso et al, 2006



Adapted from Grosso et al, 2007

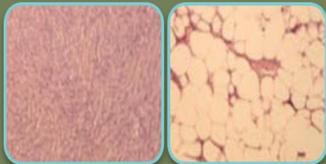
Adapted from Charytonowicz et al, 2012

Trabectedin's Complex MoA



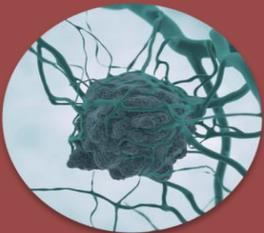
DNA Damaging

- Inducing DSB and Apoptosis
- Enhanced by BRCA deficiency, NER proficiency



Modified Transcription

- Interaction with Transcription Factors
- Inducing Adipogenic Differentiation in RC/MLPS
- Open pathway for drug combinations



Tumor Microenvironment (TME)

- Selectively targeting Monocytes and Tumor Macrophages
- Shifting TME to pro-inflammatory & Antiangiogenic profile
- TME mediated anti-tumor activity

Trabectedin's Complex MoA



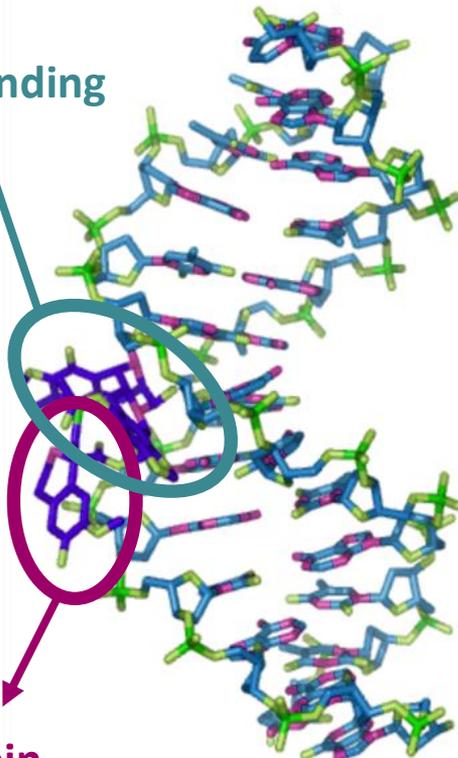
DNA Damaging

- Inducing DSB and Apoptosis
- Enhanced by BRCA deficiency, NER proficiency

Trabectedin binds to the DNA minor groove

DNA

DNA binding

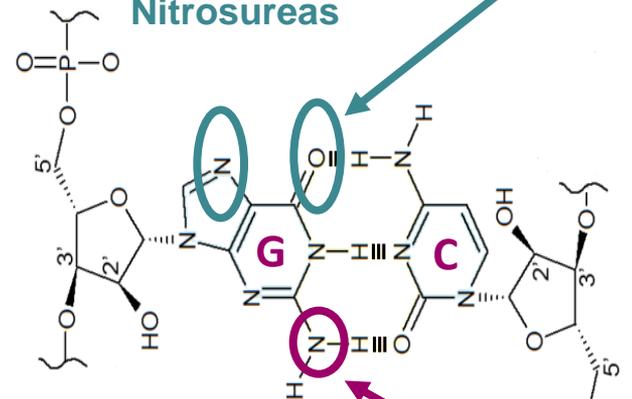


Protein binding

Major groove

Cisplatin
Melphalan
Mechlorethamine
Busulfan
Chlorambucil
Cyclophosphamide
Nitrosureas

Guanine
N7&O6



Minor groove
Trabectedin

Guanine
N2

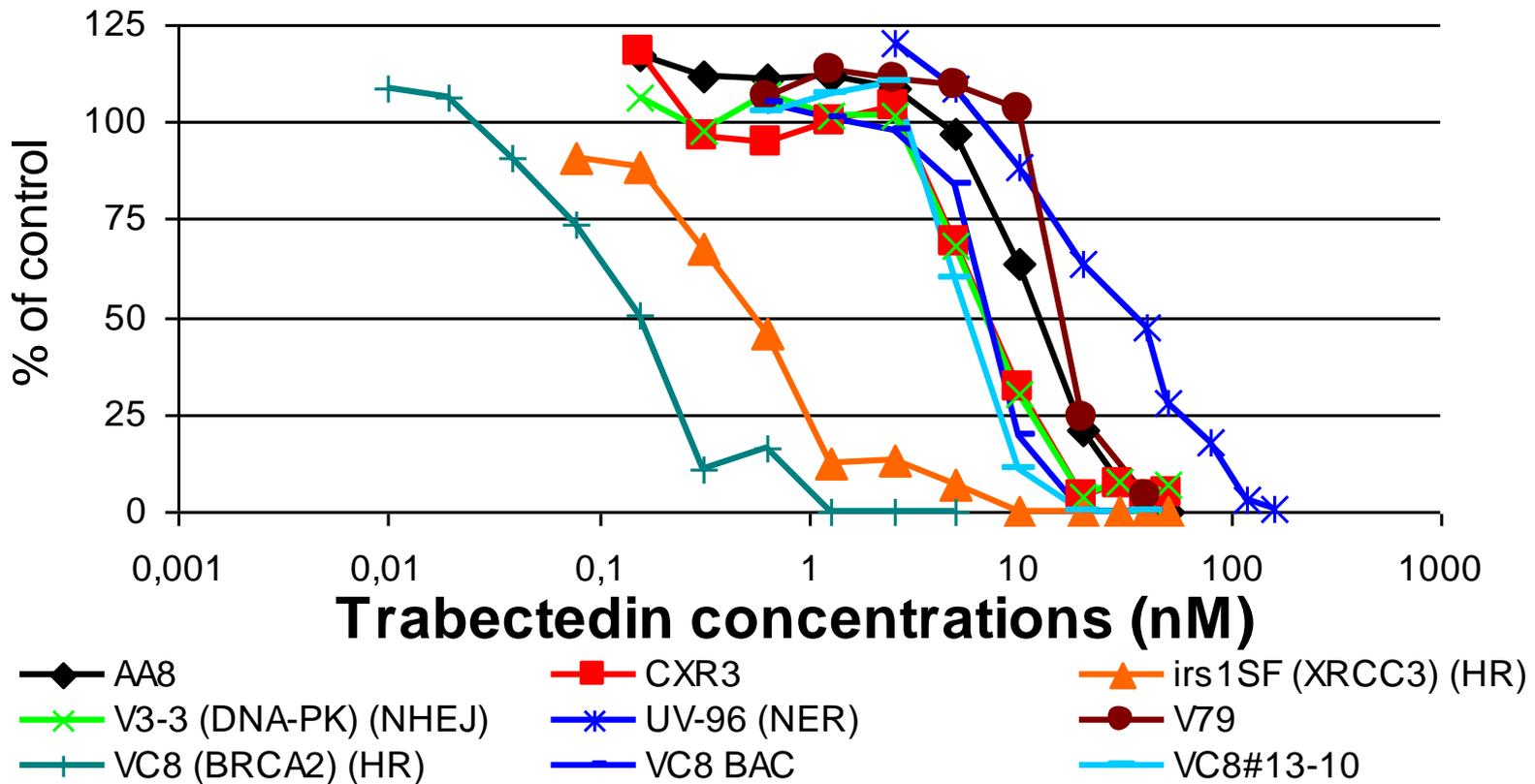
Trabectedin and DNA repair

Activity in cells defective in:

	Mismatch repair	Nucleotide excision repair	Homologous recombination repair
UV		↑↑↑↑	
γ IR			↑↑↑↑
Platinum complexes	↓	↑↑↑↑	↑↑
Trabectedin	↑/-	↓↓↓↓	↑↑↑

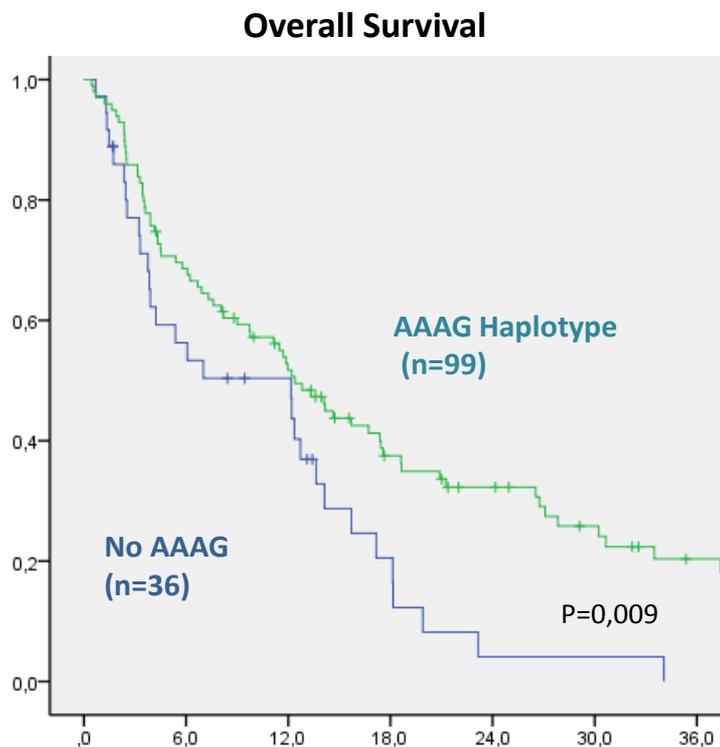
Sensitivity to Trabectedin increased by defects in HR repair

Sensitivity to trabectedin of different isogenic cell lines (colony assay)



BRCA1 haplotype may be predictive of trabectedin efficacy

- Sarcoma patients carrying BRCA1 mutation “AAAG” respond better to trabectedin.

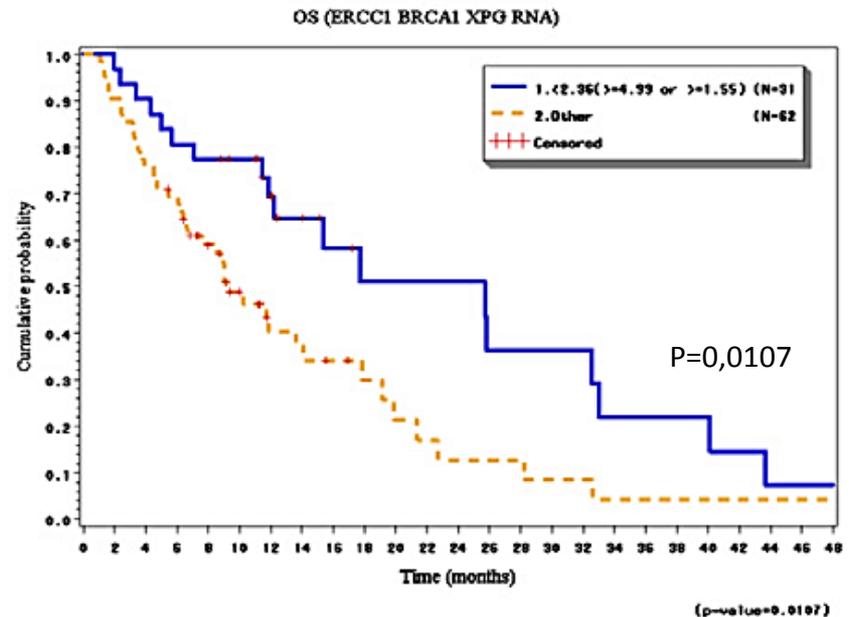
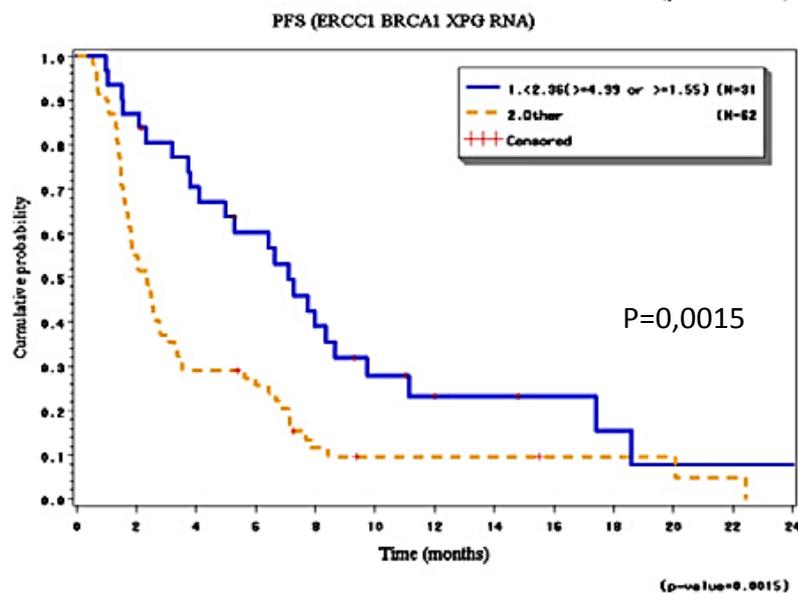


- Could be easily translated into routine clinical practice pending on results of prospective validation study.

Study Population		
	Training cohort (n=62)	Validation cohort (n=73)
Median age (years)	49	56
Range	18-74	21-78
Sex		
Male	29	29
Female	33	44
Histological subtype		
Liposarcoma	18	9
MRCL	10	4
Other	8	5
Leiomyosarcoma	18	34
Synovial sarcoma	10	11
Unclassified	10	5
Other	6	14
Histological grade		
Grade 1	3	0
Grade 2	13	19
Grade 3	32	31
Unknown	14	23

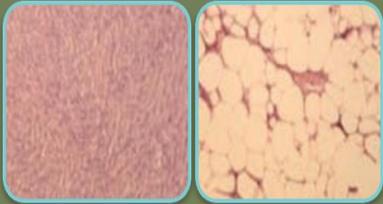
Clinical response to Trabectedin influenced by BRCA profile

- 245 tumor samples were retrospectively collected from sarcoma patients treated with single agent trabectedin in the context of a compassionate use program
- Significant differences in PFS and OS detected for patients with high XPG and low BRCA1 expression treated with trabectedin
- BRCA1 deficiency and XPG proficiency enhanced clinical responses to trabectedin



Trabectedin's Complex MoA

Modified Transcription



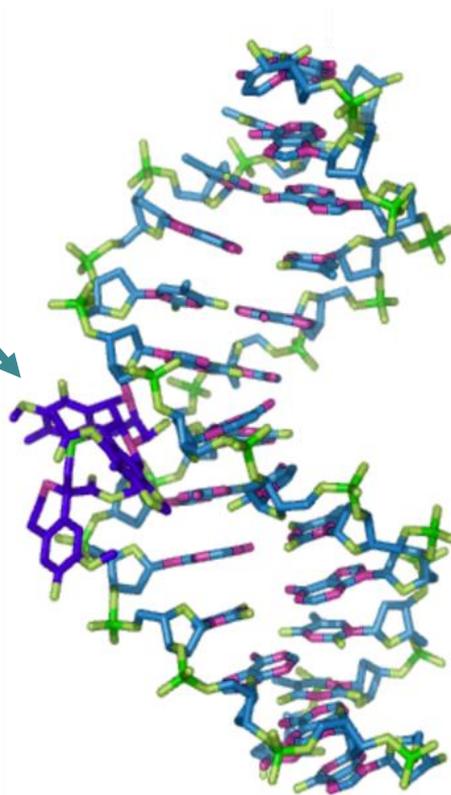
- Interaction with Transcription Factors
- Inducing Adipogenic Differentiation in RC/MLPS
- Open pathway for drug combinations

Displacement of proteins from DNA

HMGA
SRF
E2F1
NF-Y

Transcription
Factors

EWS-FLI1
FUS-CHOP



Modulation of the expression
of downstream targets

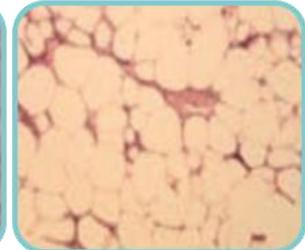
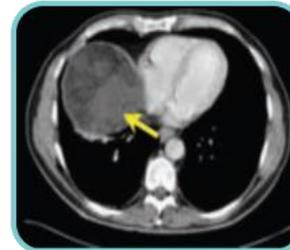


Changes in tumor biology

Resumption of terminal
differentiation processes

Pre-treatment

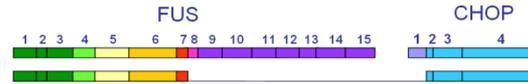
Cycle 2



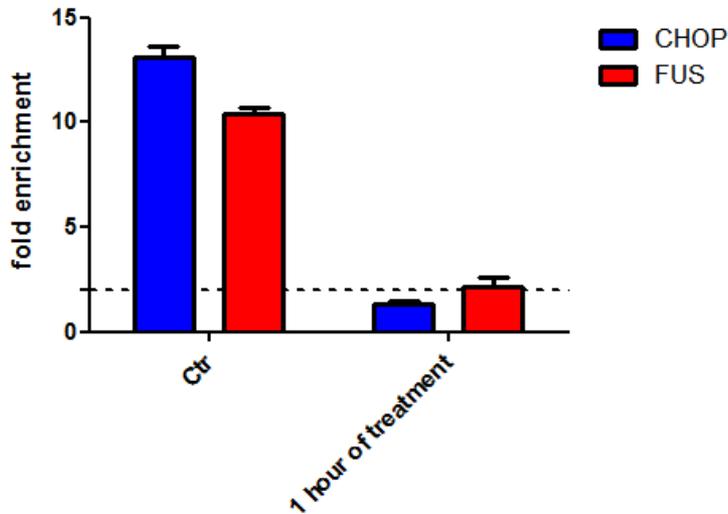
Trabectedin causes the detachment of FUS-CHOP from the promoter regions of its target genes in *in vitro* models

402-91 cell line

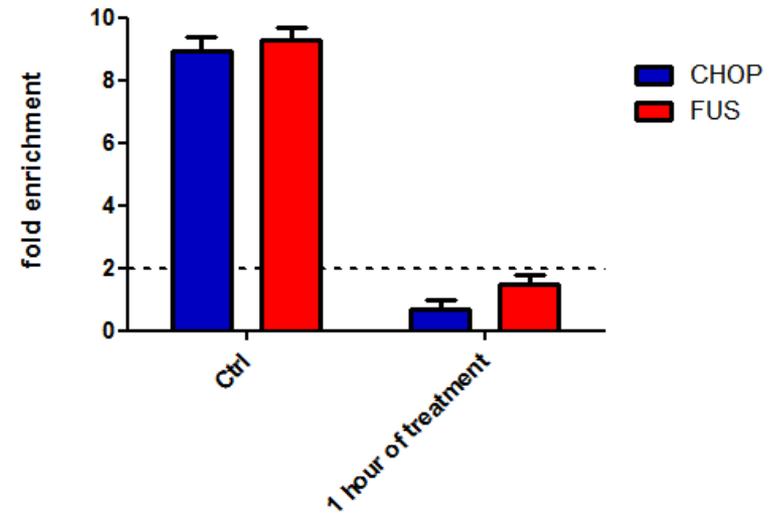
Type I



PTX3



FN-1



ChIP from untreated cells and treated with 2nM of trabectedin for 1 hour with α -CHOP, α -FUS, α -Flag (Ctrl) antibodies. Two promoters were evaluated in quantitative Real Time PCR analysis. Values are reported as fold enrichment over anti-Flag antibody.

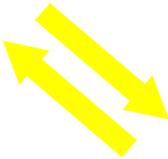
OPERATING THEATRE



PATHOLOGICAL CLASSIFICATION



SHORT TERM
CULTURES



MOLECULAR INVESTIGATION
FISH, RT-PCR,
DNA sequencing



MOUSE
TRANSPLANTATION

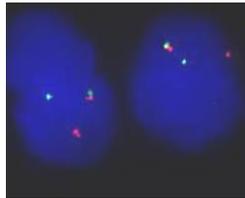
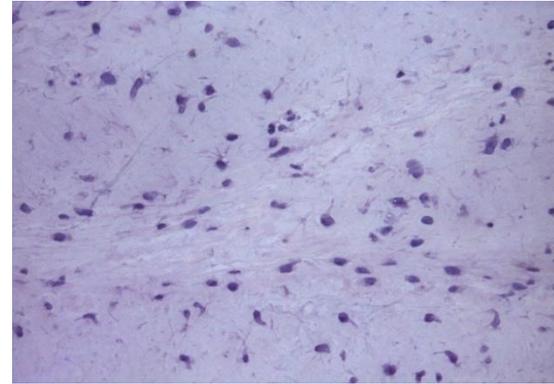
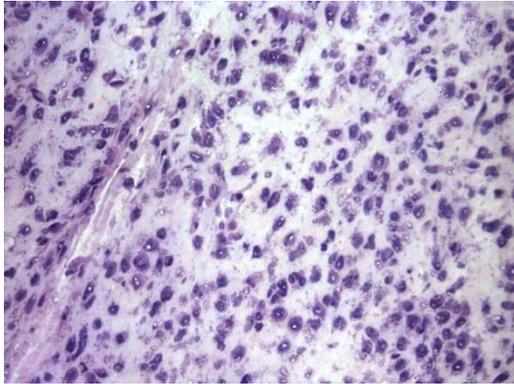


Pre-treatment

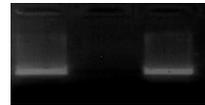
Post-treatment 6 doses of ET

A.

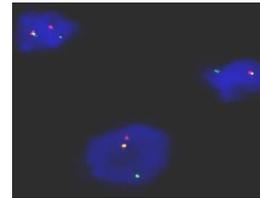
Xenograft
(4°P ML017)



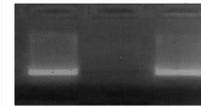
c+ c- PR



FUS-CHOP
Type II



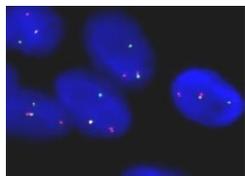
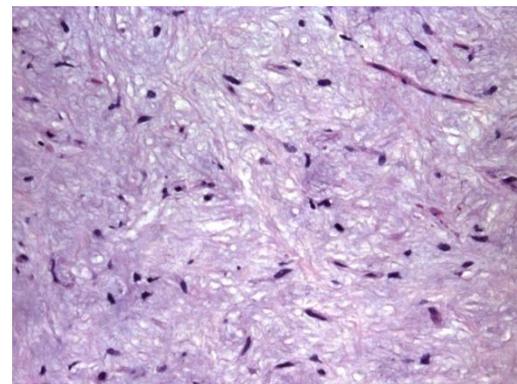
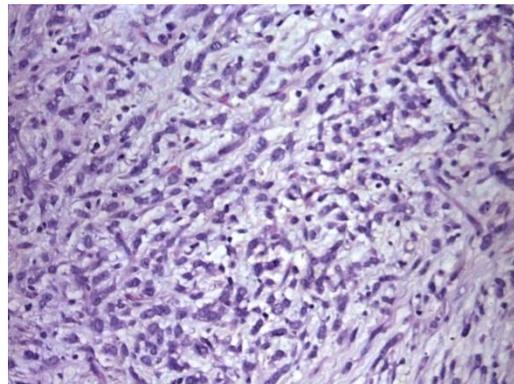
c+ c- PR



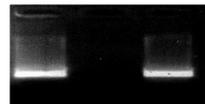
FUS-CHOP
Type II

B

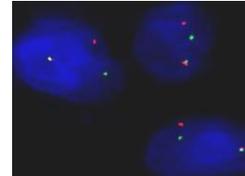
Human
Surgical
Specimen



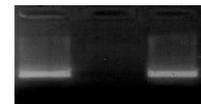
c+ c- PR



FUS-CHOP
Type II



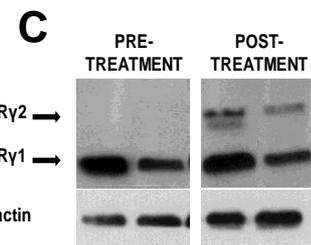
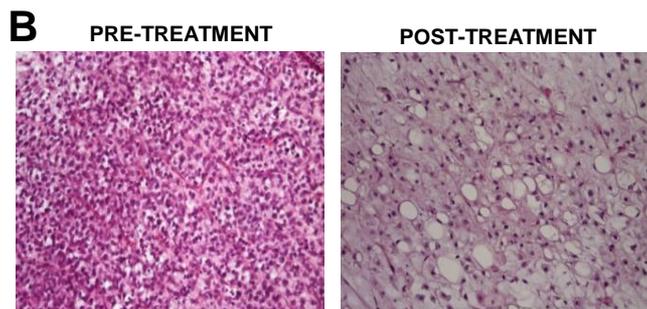
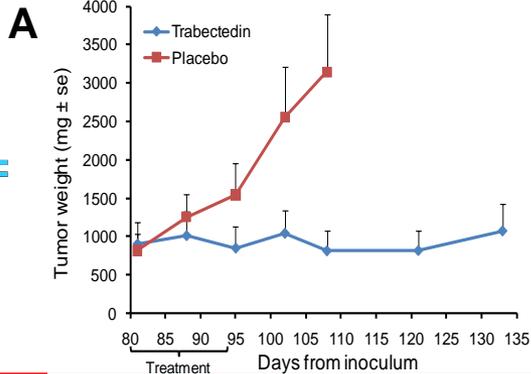
c+ c- PR



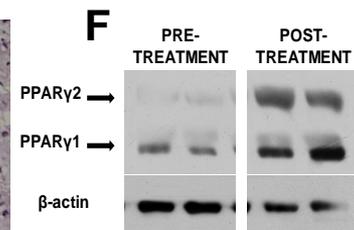
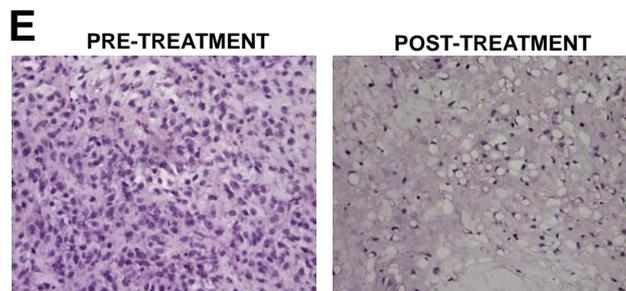
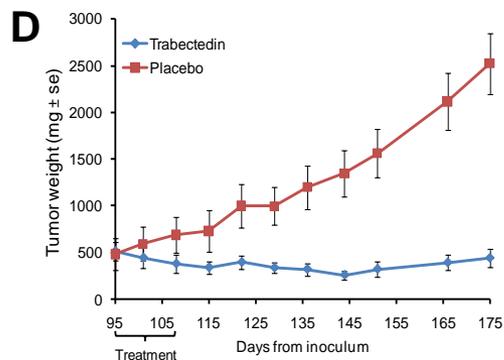
FUS-CHOP
Type II

Trabectedin induces antitumor response associated to adipocytic maturation and antiangiogenic effects in type I/II, but not in type III myxoid liposarcoma

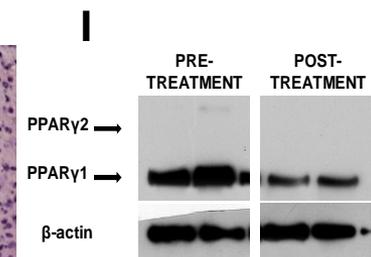
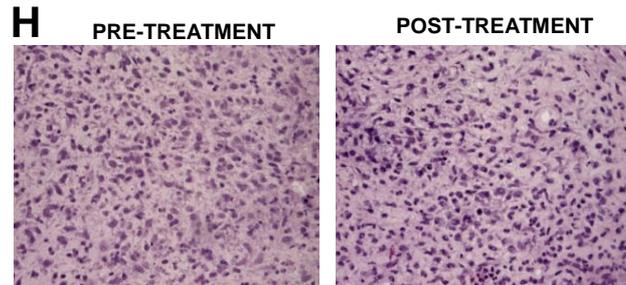
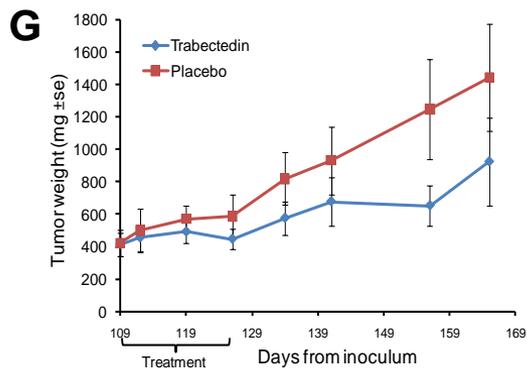
Type I



Type II

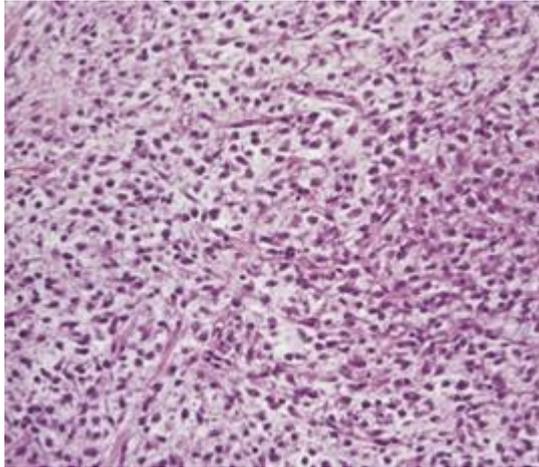


Type III

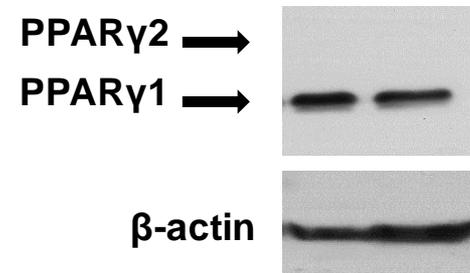
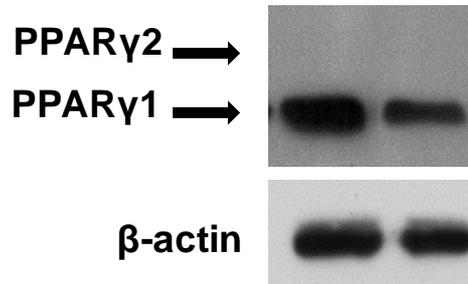
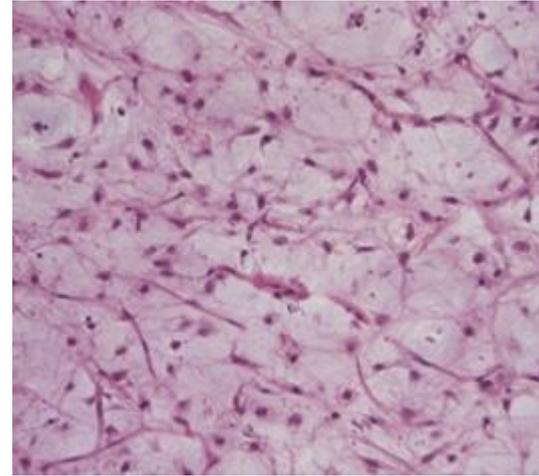


Doxorubicin is very effective, but PPAR γ 2 (marker of adipocytic differentiation) is not induced and the vascular effect is not as evident as that of trabectedin

Pre-Treatment

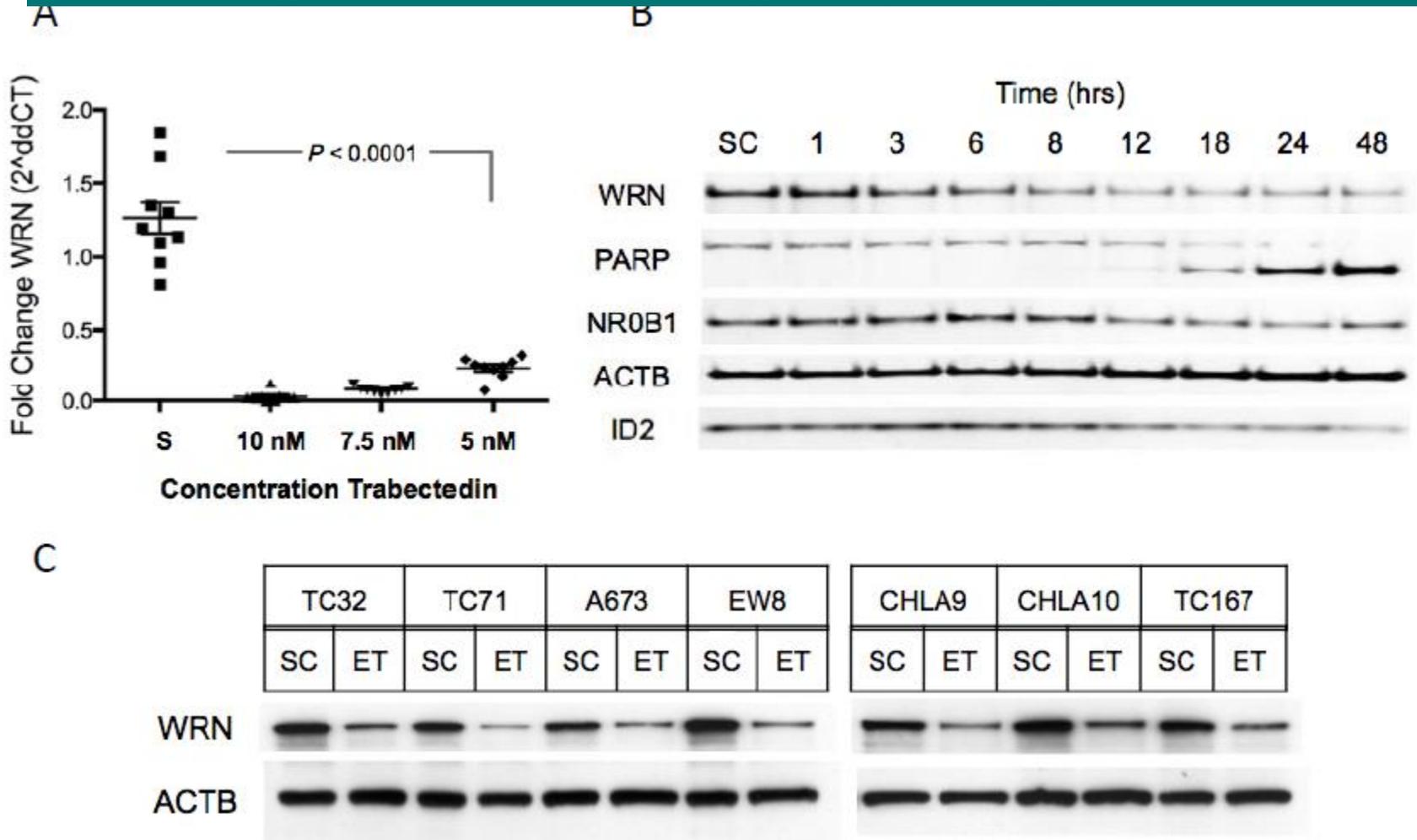


Post-Treatment



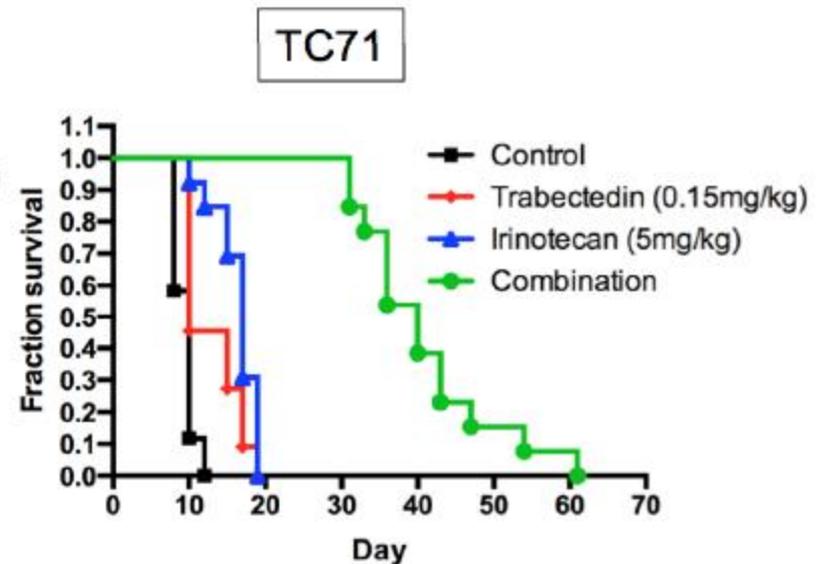
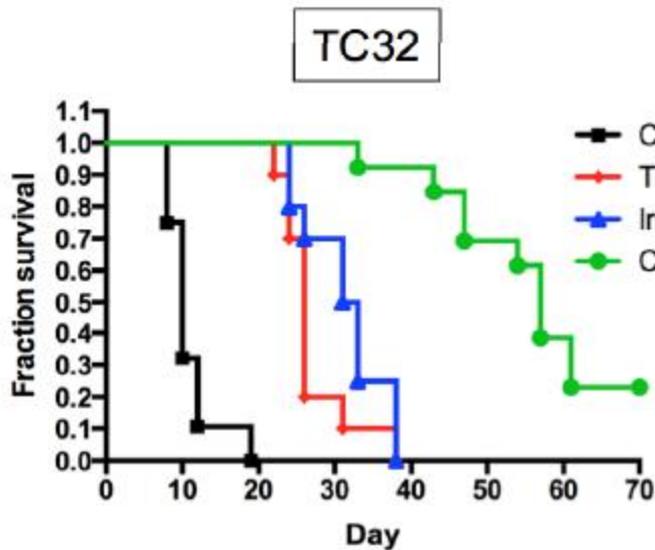
Trabectedin shows the **unique property** to displace an oncogenic transcription factor from its target promoters in a selective fashion

Trabectedin causes downregulation of Werner syndrome (WRN) gene by inhibiting EWS-FLI1 transactivating ability in Ewing sarcoma cells

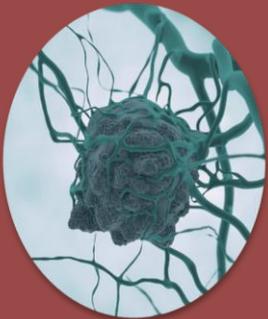


It is known that cells deficient in WRN syndrome are very sensitive to topoisomerase I inhibitors.

The sequential treatment of trabectedin and irinotecan is a highly effective regimen in Ewing sarcoma xenografts regardless their sensitivity to each drug given alone



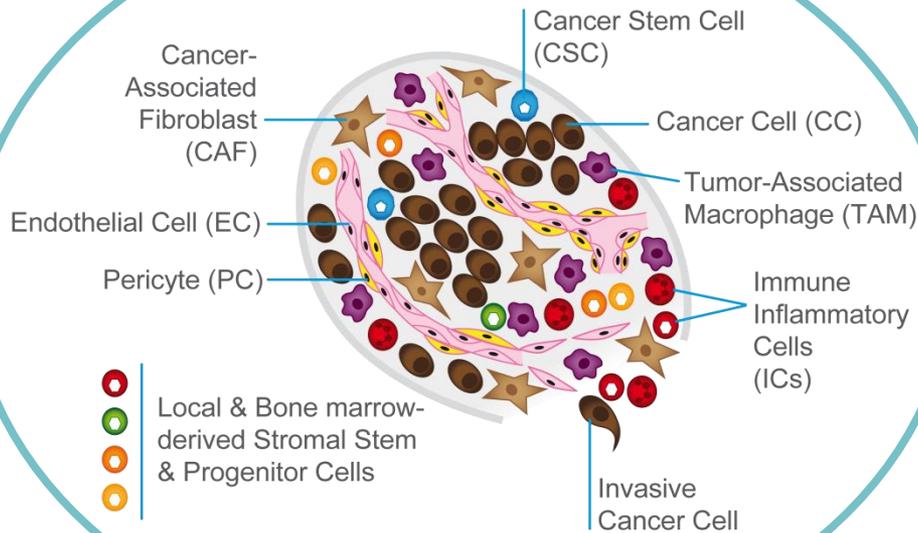
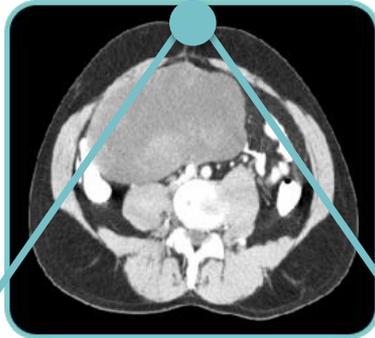
Trabectedin's Complex MoA



Tumor Microenvironment (TME)

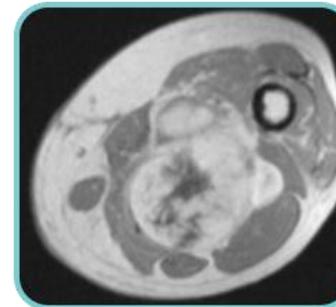
- Selectively targeting Monocytes and Tumor Macrophages
- Shifting TME to pro-inflammatory & Antiangiogenic profile
- TME mediated anti-tumor activity

Tumors are organ-like structures

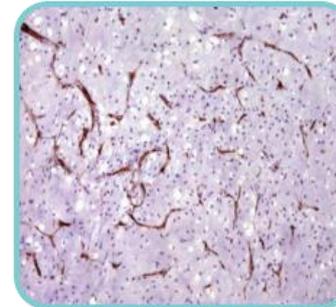
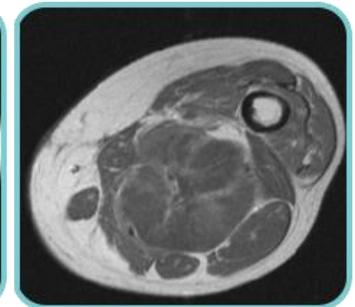


Disappearance of vascular pattern after Trabectedin

Pre-treatment

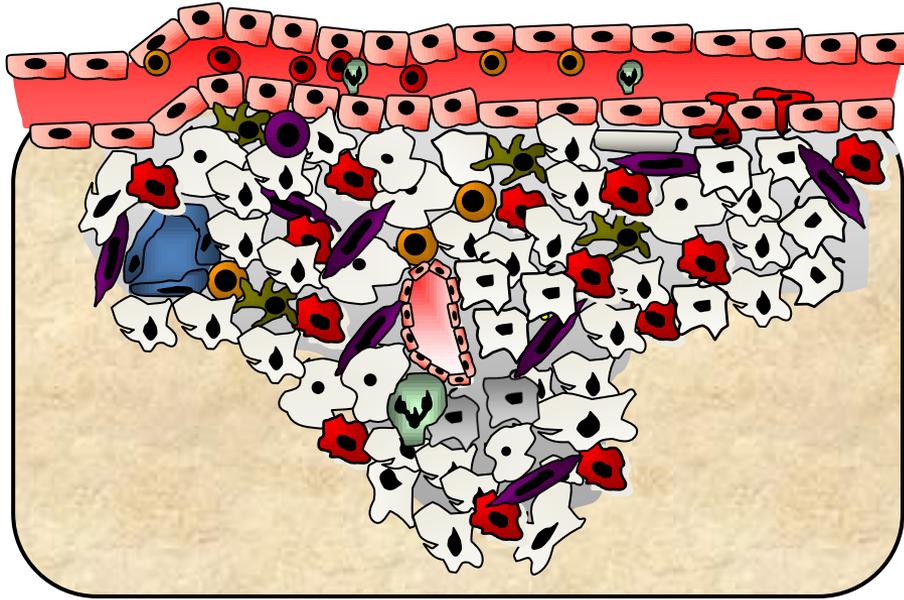


Cycle 4



Immunostaining for CD31

TAM early and relevant inflammation promoters



- Tumor cell survival/proliferation
- Tumor cell invasion
- Angiogenesis
- Matrix remodeling
- Immune suppression



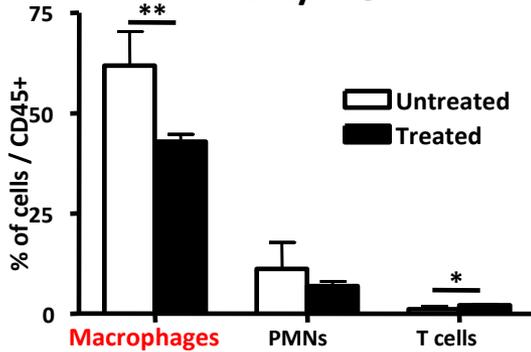
The number of Tumor-Associated Macrophages (TAM) significantly correlates with tumor progression in a number of human tumors:

- Advanced tumor stage
- Shorter disease-free survival
- Resistance to chemotherapy and anti-angiogenic therapy

TAM are decreased after trabectedin

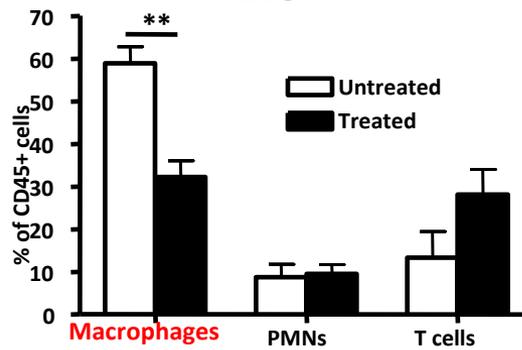
Fibrosarcoma Cells (mouse)

MN/MCAI



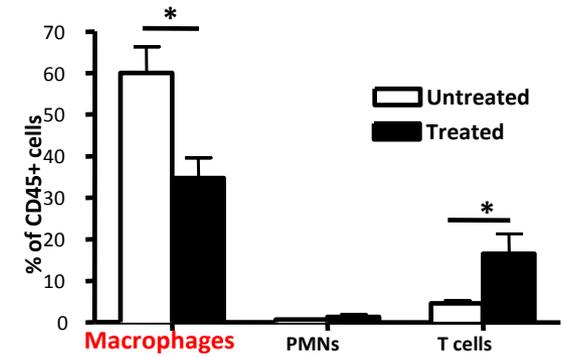
Lewis Lung Cells (mouse)

LLC



Ovarian Cancer Cells (mouse)

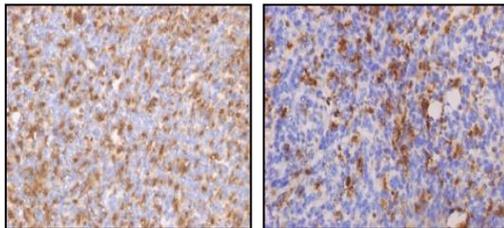
ID8



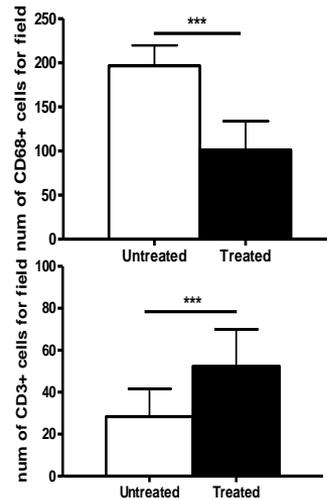
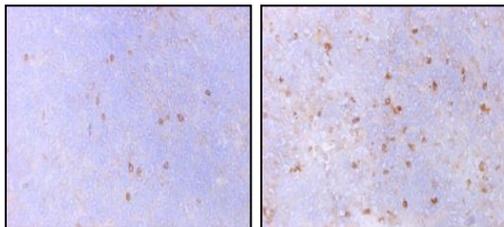
Untreated

Treated

CD68



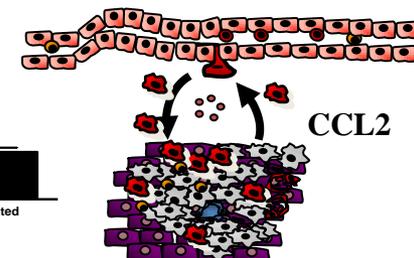
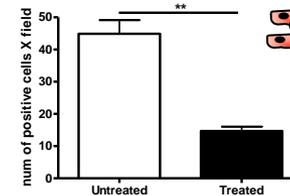
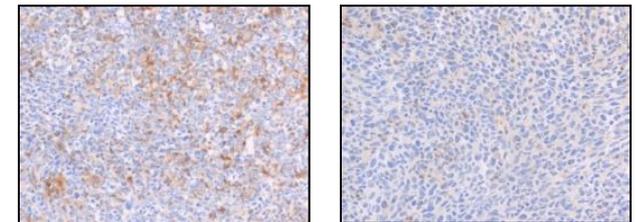
CD31



Untreated

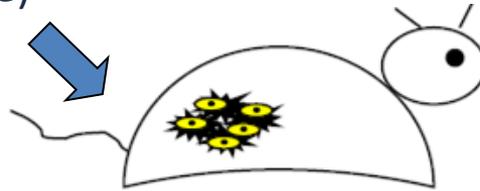
Treated

CCL2



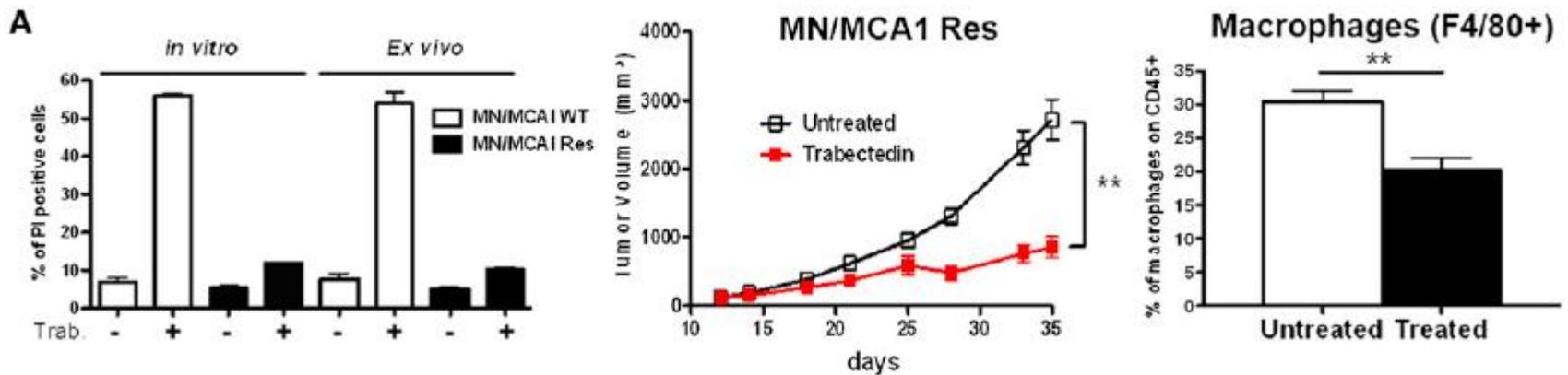
In vivo effect on tumor microenvironment

Trabectedin resistant fibrosarcoma cells (mouse)

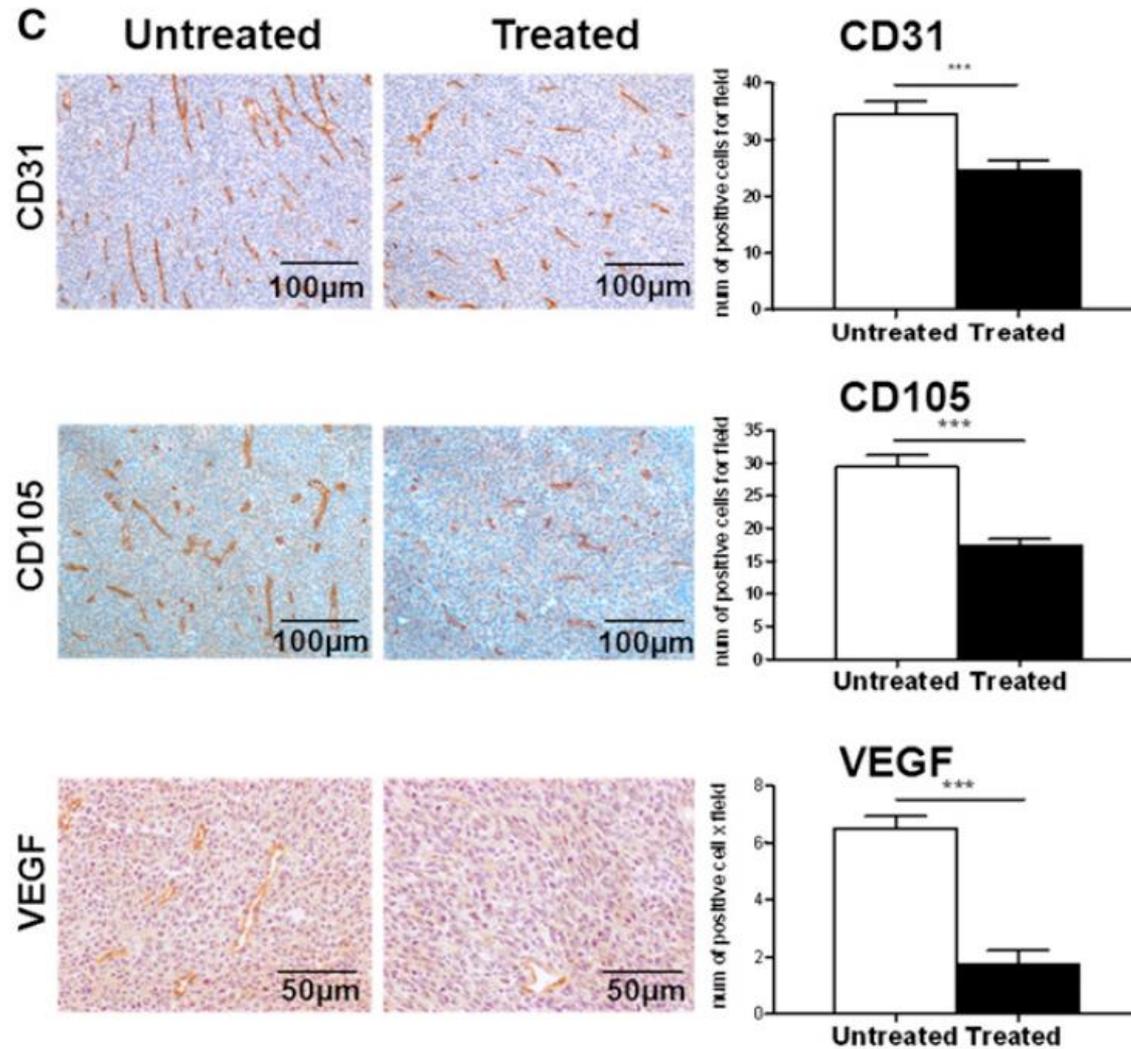


Tumors originated in mice using cells resistant to trabectedin

Cancer cells remain resistant, but the tumor responds to trabectedin in vivo



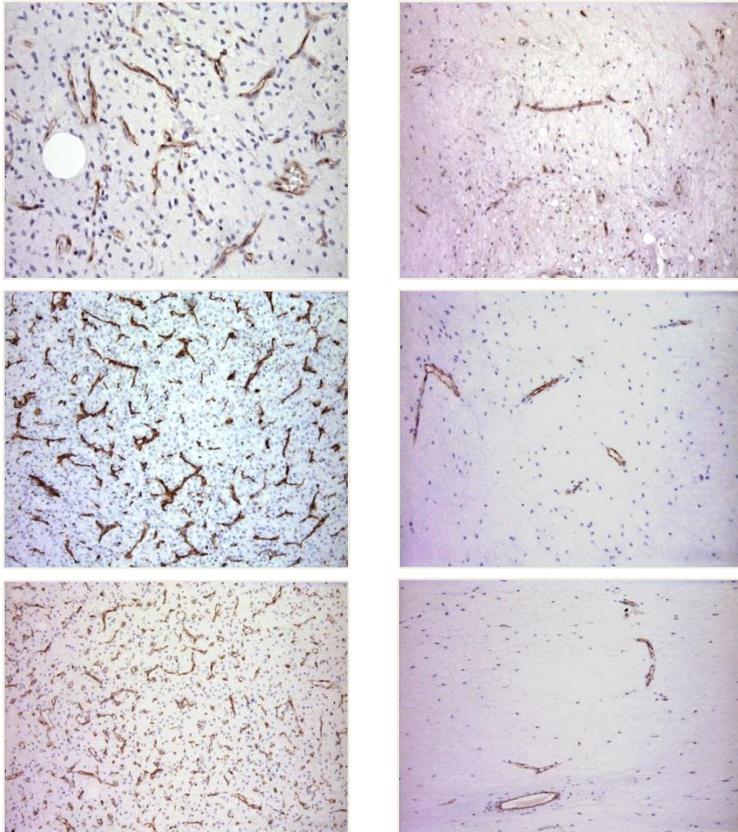
Decreased angiogenesis in trabectedin-treated tumors



TAM and vessels are reduced in treated STS patients

PRE*

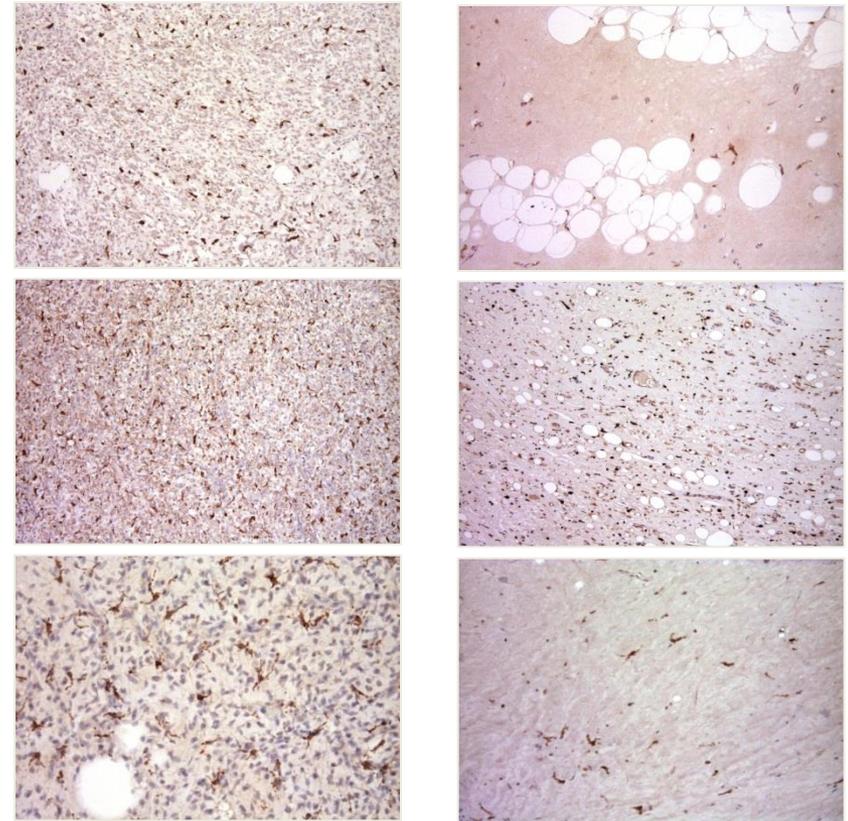
POST**



CD31 vessels

PRE*

POST**

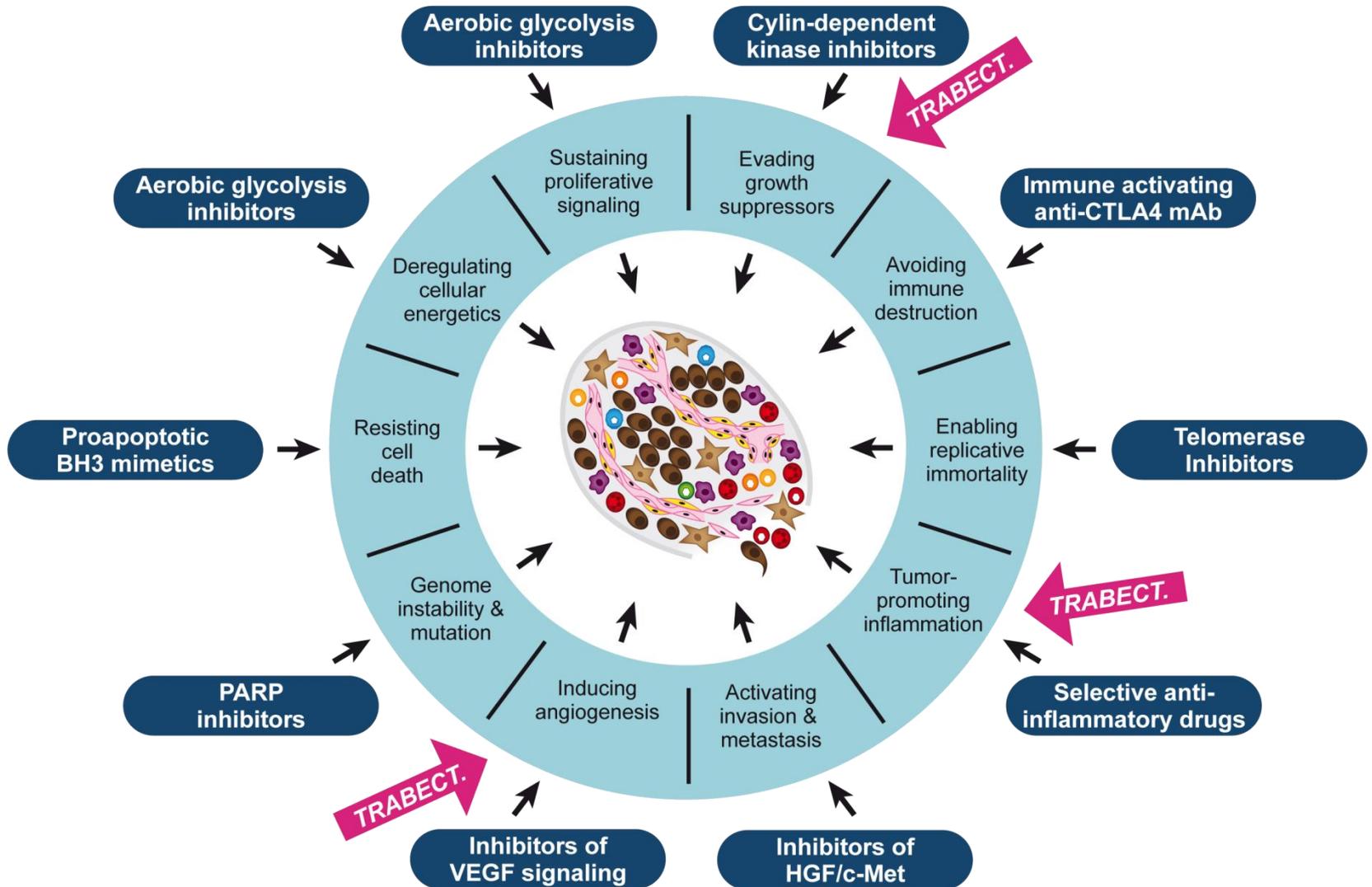


CD163 macrophages

* PRE: biopsy before surgery;

** POST: tumor sample at surgery, after therapy

Multifunctional therapeutic targets



Adapted from Hanahan & Weinberg, Cell 2011

Conclusions

- Trabectedin is the first compound that is able to target an oncogenic transcription factor with high selectivity.
- The antitumor activity of trabectedin seems to be related to **direct effects on cancer cells** with induction of growth inhibition cell death and differentiation and **indirect effects related to its anti-inflammatory and anti-angiogenic** properties.
- The effects on tumor **microenvironment** are in keeping with the pattern of response observed in several patients, i.e. a delayed response **with a prolonged stabilization** (*tumor dormancy*).
- Studies are in progress to define whether and at which extent the biological characterization of the tumors will allow to select patients who can benefit more from trabectedin treatment, alone or in combination with other drugs.

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