Daniel Rodríguez Ajamil

ESR13 Fellow

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Meeting 2
Edinburgh, 3rd February 2020

Innovative Training Networks (ITN) – Marie Skłodowska-Curie Actions
H2020-MSCA-ITN-2017 (THERACAT-765497)

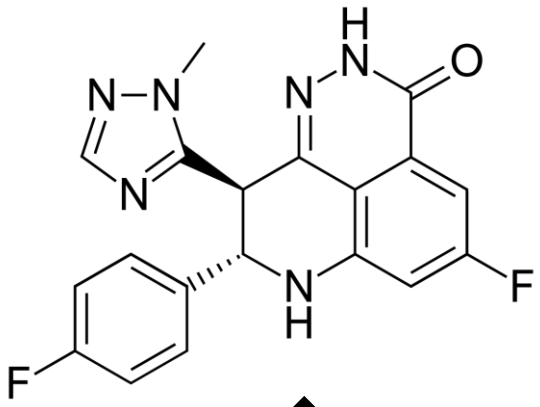
Bioorthogonal Catalysis for BRCA Mutated Breast Cancer

HR-/HER2- – – – – Aka "Triple Negative (TNBC)"

15% of all breast cancers

- Difficult to target therapeutically
- Current therapy: surgical resection and removal of auxiliary lymph nodes, with postoperative systemic chemotherapy.
- **1/3 of TNBC carry BRCA1 or BRCA 2 mutations**

PARPi



Defective DNA repair mechanisms

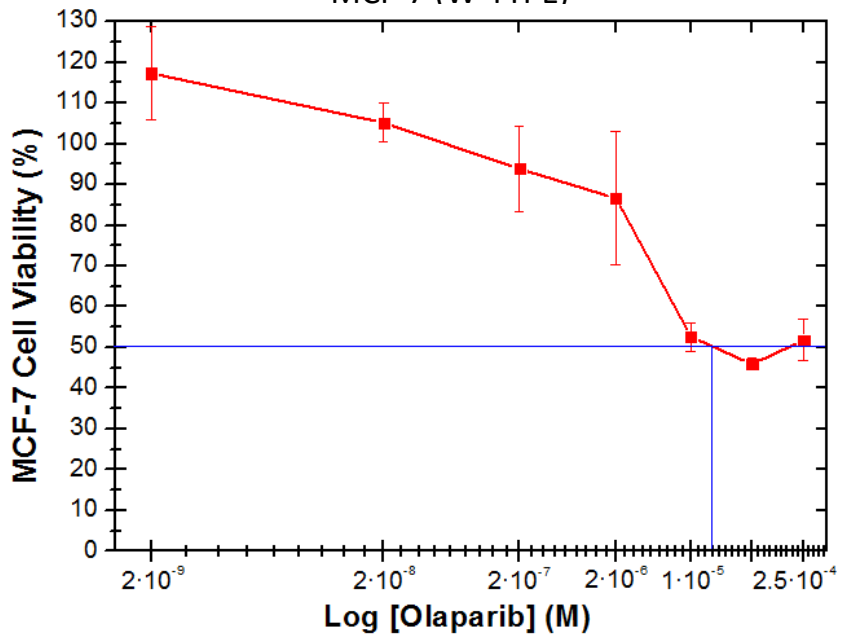
Increases 70% cancer prob.

Therapeutic Opportunity: Synthetic Lethality

Results obtained

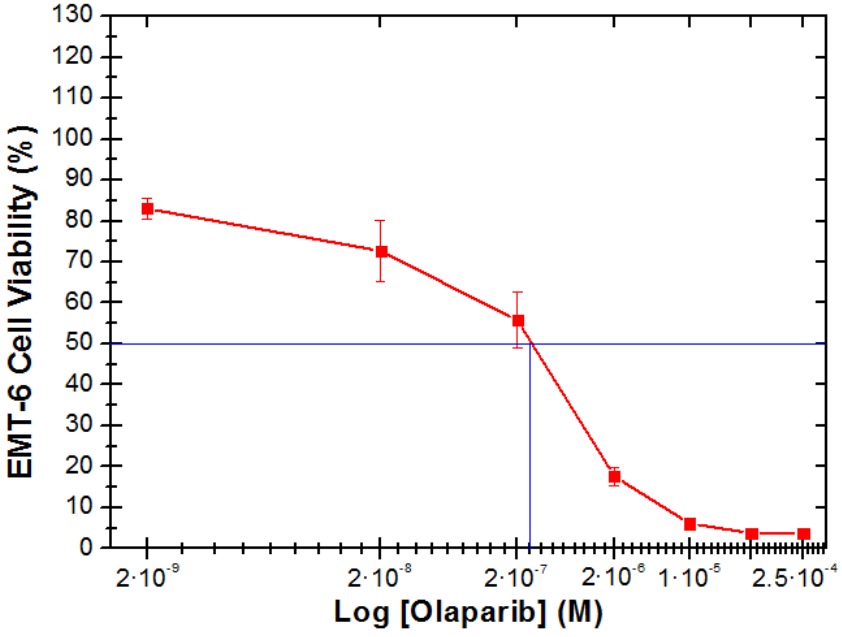
OLAPARIB (Anti-Proliferation effect)

MCF-7 (W-TYPE)



IC50 ca. 400 uM

EMP-6 (BRCA MUTATED)

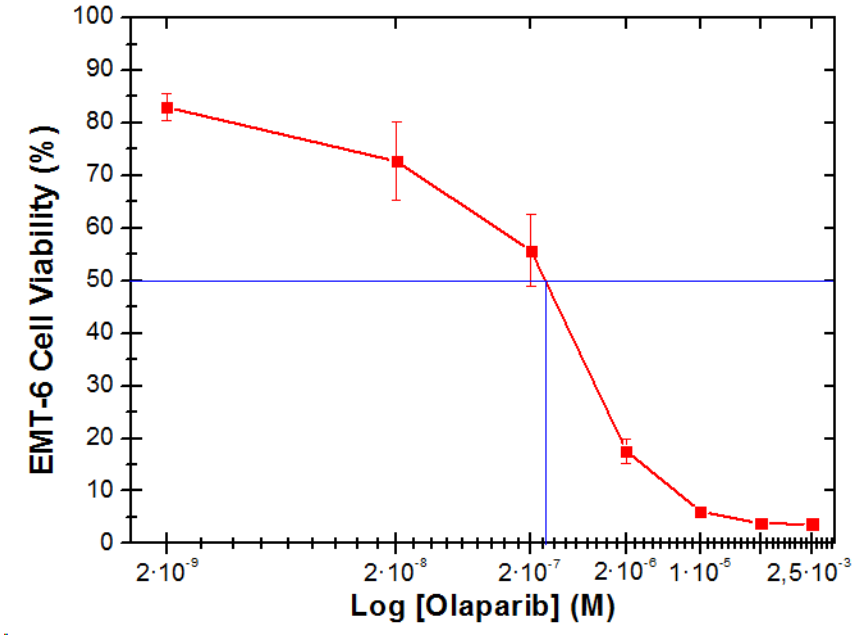


IC50 ca. 1 uM

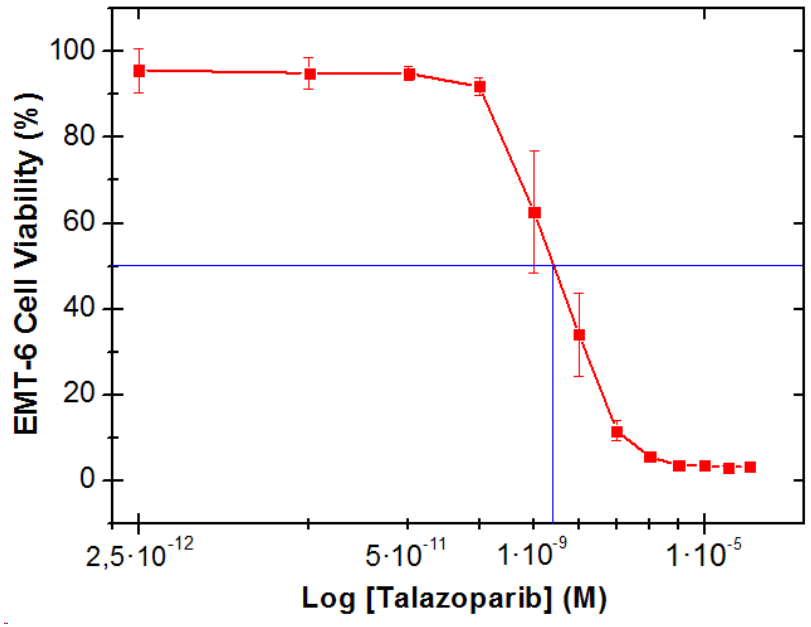
N=3

Results obtained

OLAPARIB VS. TALAZOPARIB



IC50 ca. 1 uM

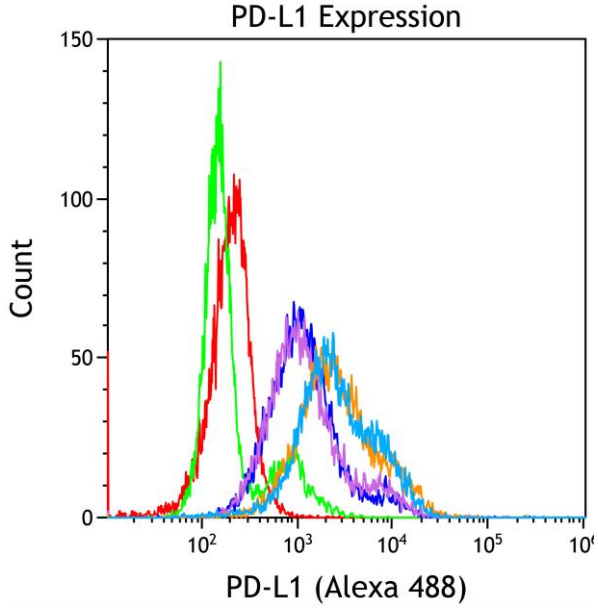


IC50 ca. 1,2 nM

N=3

Results obtained

EMT-6 cells PD-L1 expression under Talazoparib treatment

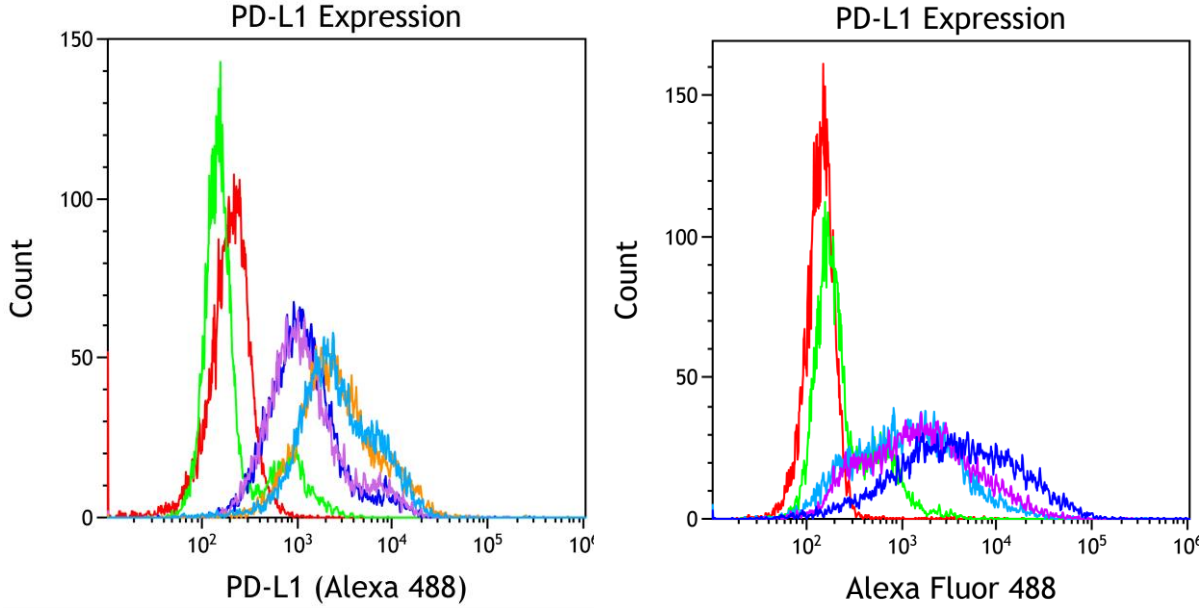


Marker	X-Med
Tal. 1uM	2,421.67
Tal. 10nM	2,421.06
Tal. 0.1nM	1,084.52
Control	1,096.83
Sec. Only	163.63
Unstained	204.34

- EMT6 cells have high basal expression of PD-L1
- PD-L1 expression increases with the concentration of the Talazoparib treatment

Results obtained

EMT-6 cells PD-L1 expression under Talazoparib treatment



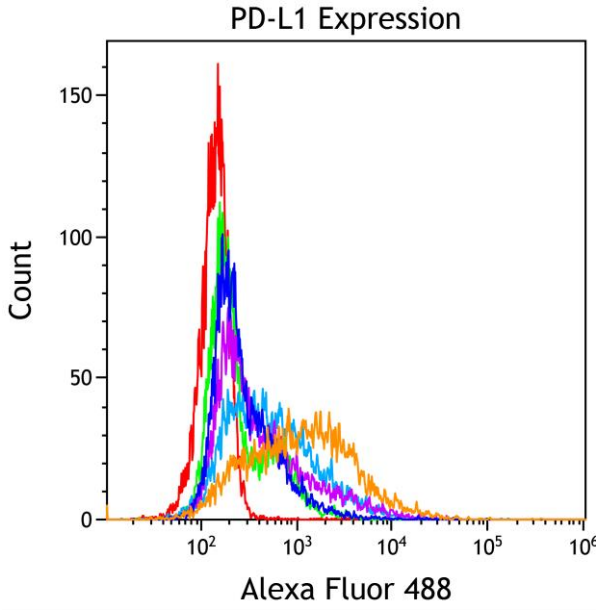
Marker	X-Med
Tal. 1uM	2,421.67
Tal. 10nM	2,421.06
Tal. 0.1nM	1,084.52
Control	1,096.83
Sec. Only	163.63
Unstained	204.34

Marker	X-Med
Tal 72h	3,715.03
Tal 48 h	1,533.04
Control	1,137.67
Secondary only	200.59
Unstained	143.44

- EMT6 cells have high basal expression of PD-L1
- PD-L1 expression increases with the concentration of the Talazoparib treatment
- PD-L1 expression increases with the temporal exposure to Talazoparib

Results obtained

EMT-6 cells PD-L1 expression under ID56 treatment

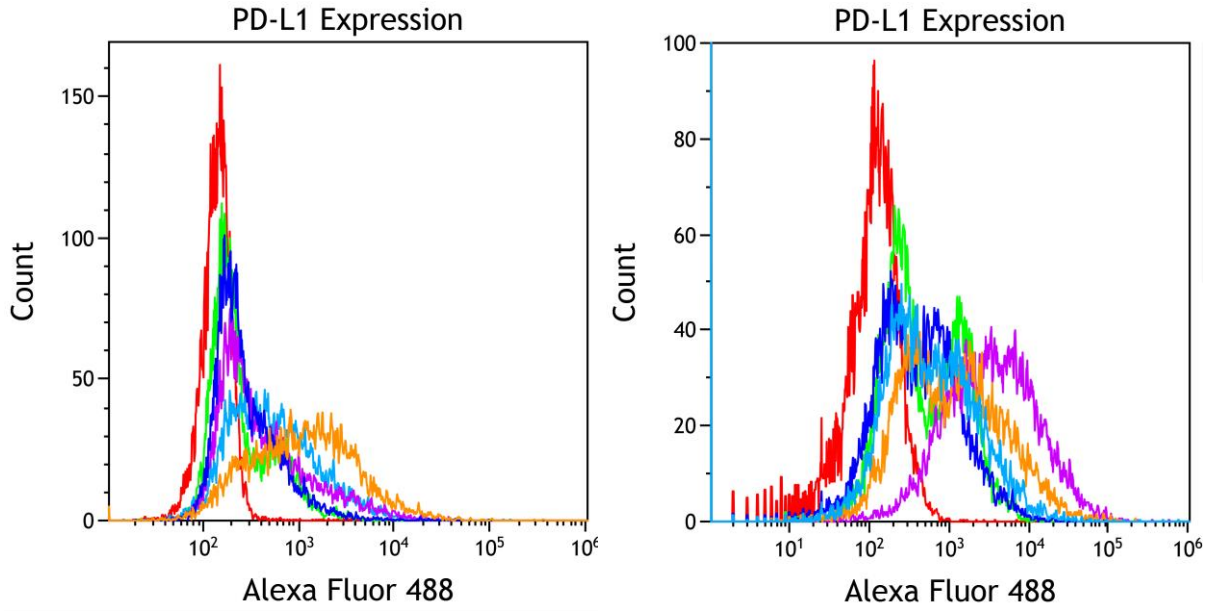


Marker	X-Med
anti PD-L1 (ID56) 72h	1,137.67
anti PD-L1 (ID56) 48h	233.92
anti PD-L1 (ID56) 24h	308.41
Control	478.40
Secondary only	200.59
Unstained	143.44

- PD-L1 signal decreases with the Treatment of ID56 (1uM)
- After 72h the expression of PD-L1 drastically increases.

Results obtained

EMT-6 cells PD-L1 expression under ID56 treatment



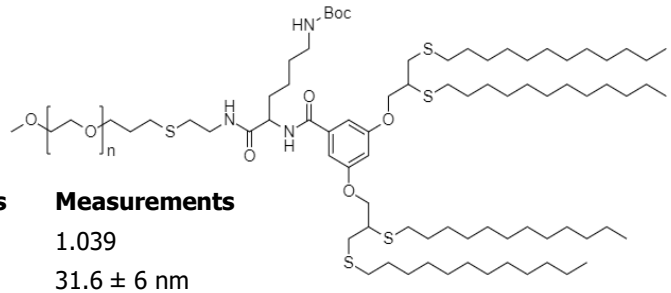
Marker	X-Med
anti PD-L1 (ID56) 72h	1,137.67
anti PD-L1 (ID56) 48h	233.92
anti PD-L1 (ID56) 24h	308.41
Control	478.40
Secondary only	200.59
Unstained	143.44

Marker	X-Med
ID56 48h	519.35
Tal 10nM + ID56 48h	973.11
Tal 10nM 48h	3,352.30
Control	381.12
Sec. Only	372.65
Unstained	116.86

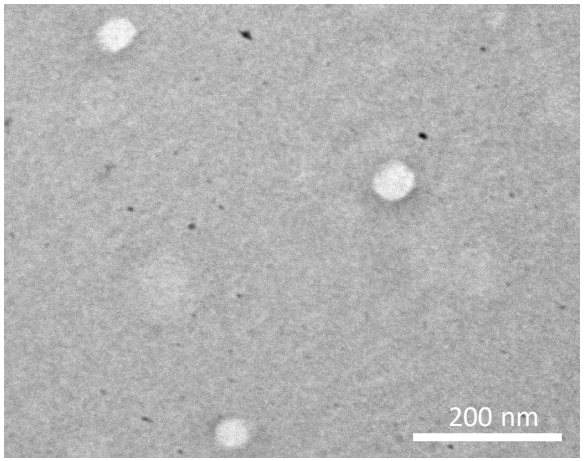
- PD-L1 signal decreases with the Treatment of ID56 (1uM)
- After 72h the expression of PD-L1 drastically increases.
- PD-L1 signal decreases with the Treatment of ID56 (1uM) also when is combined with Talazoparib (10nM)

C12-Talazoparib PEG Dendron Hybrids

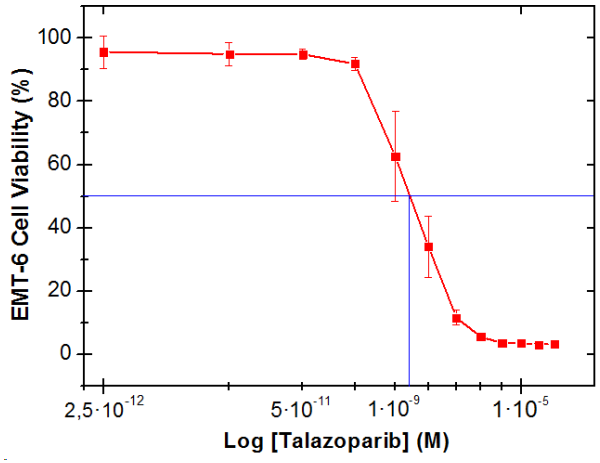
PEG-C12 Hybrid



Tests	Measurements
GPC	1.039
DLS	31.6 ± 6 nm
CMC	3±1 µM

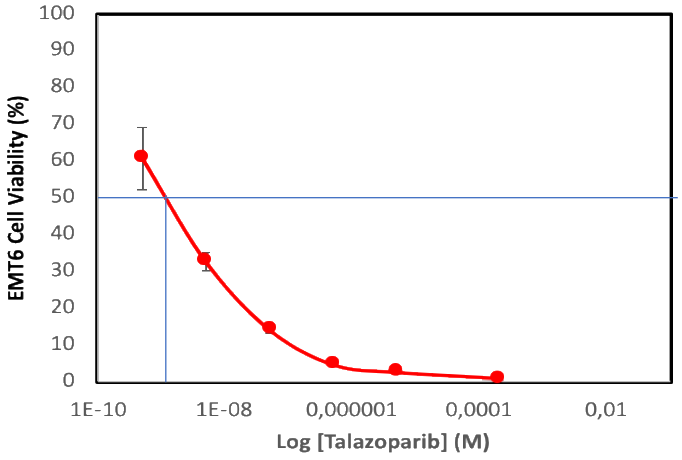


Free Talazoparib



IC50 ca. 1,2 nM

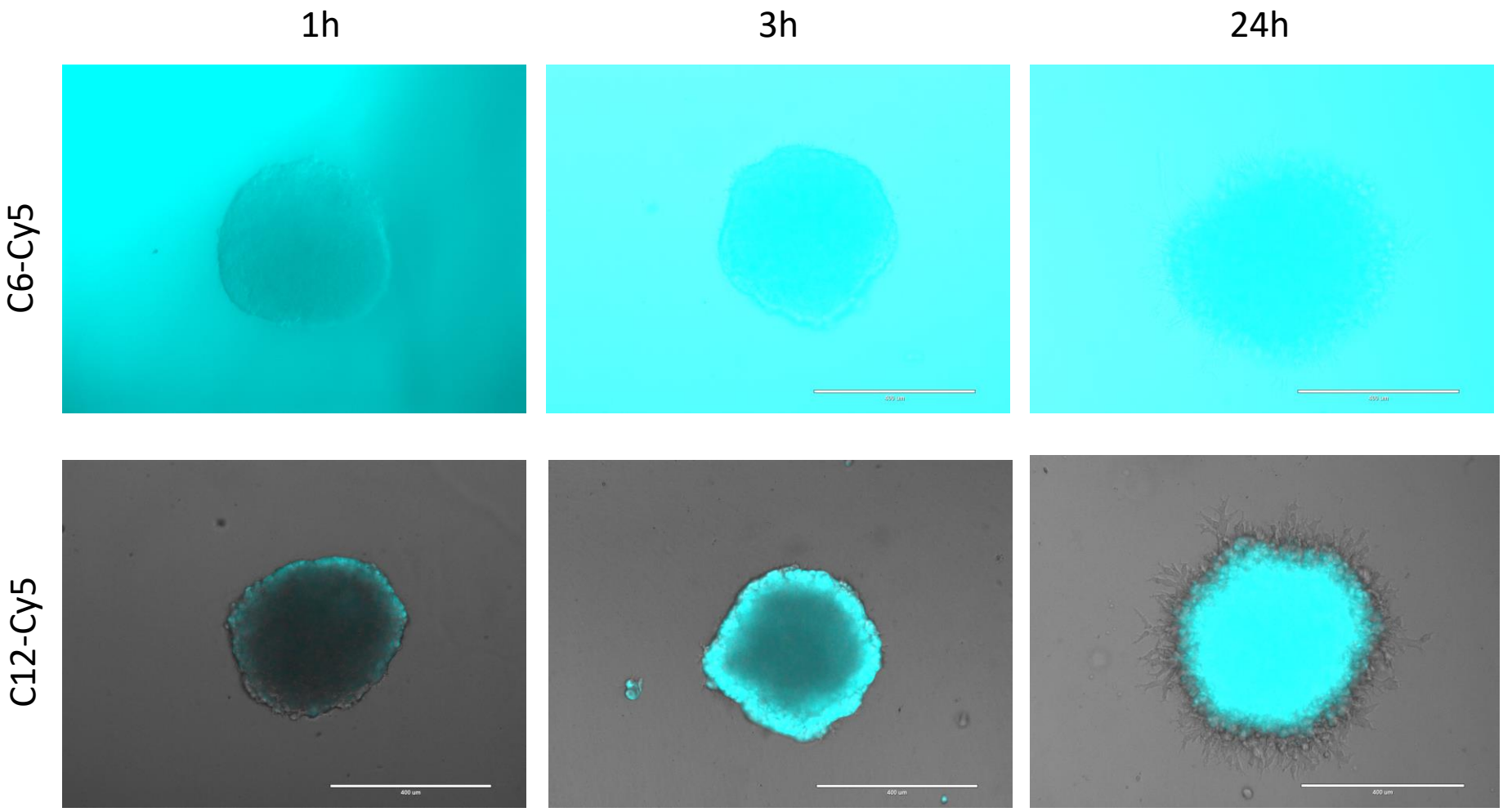
C12-Talazoparib



IC50 ca. 2 nM

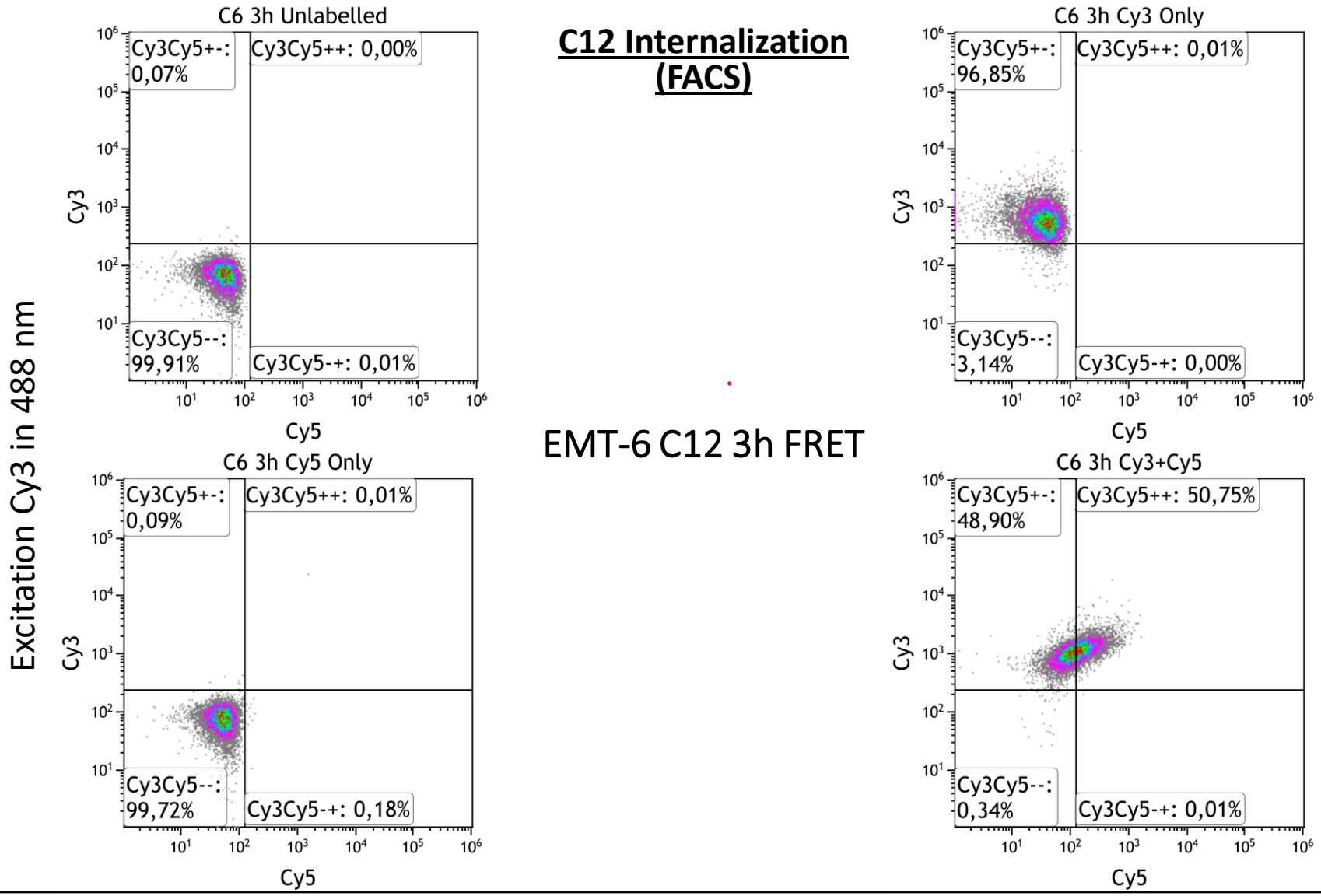
Results obtained

C12 Internalization in EMT6 3D Spheroids



Results obtained

C12 Internalization (FACS)



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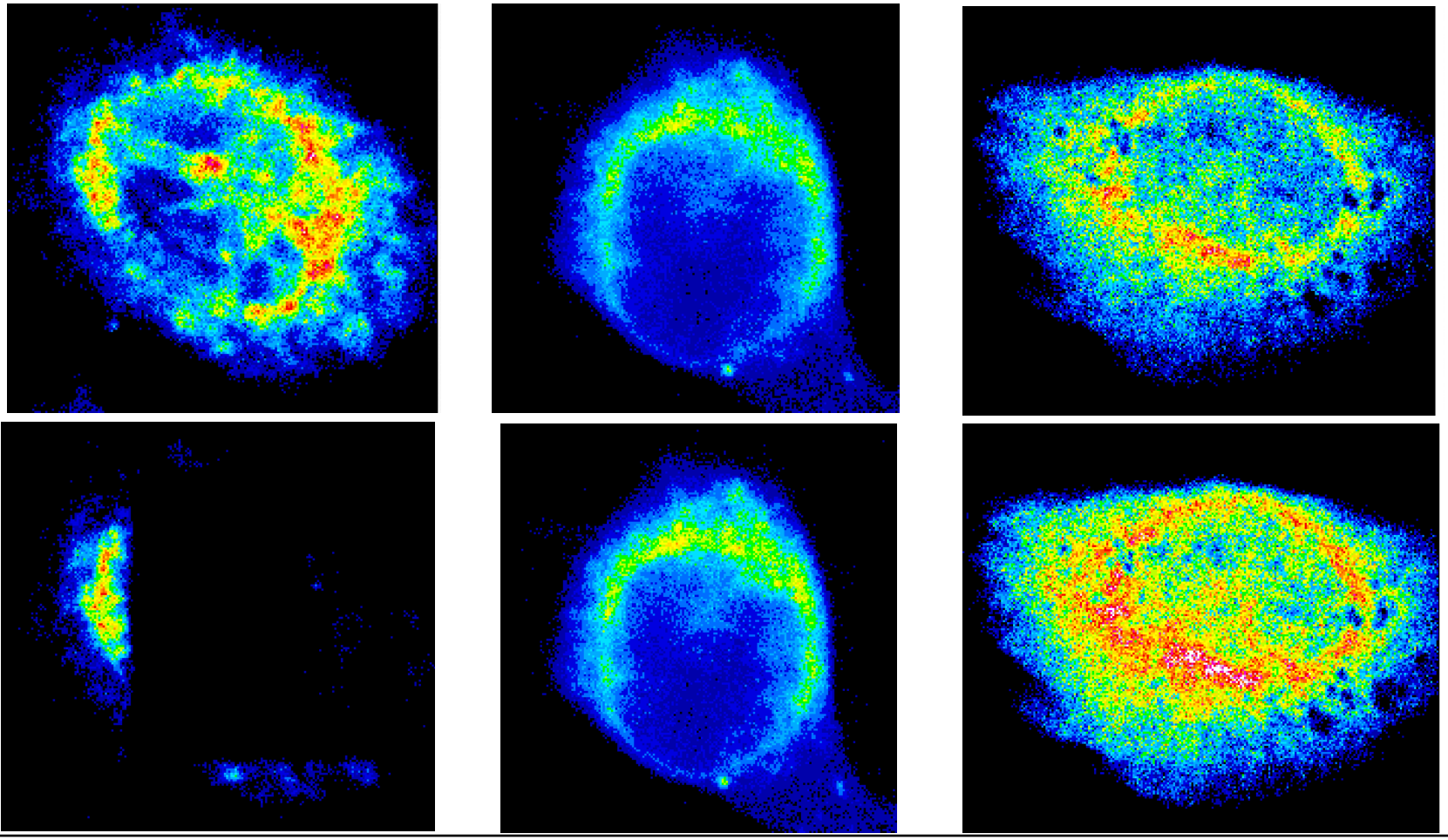
Results obtained

C12 Internalization (Confocal)

FRET Micelles (Cy5 Channel) | Cy3 Micelles (Cy3 Channel) | FRET Micelles (Cy3 Channel)

Before Cy5 Bleaching

After Cy5 Bleaching



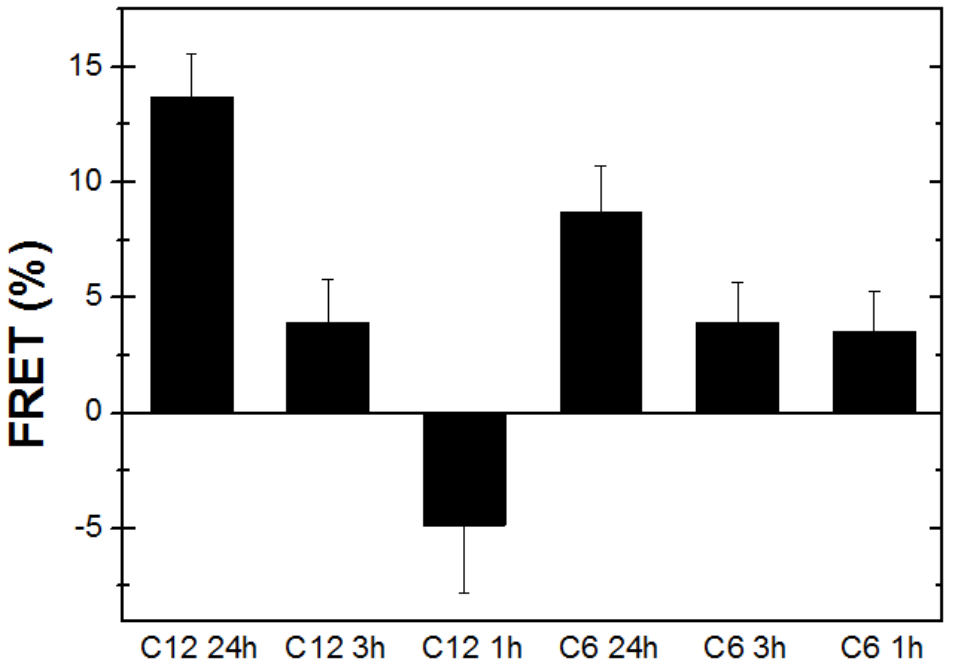
Results obtained

C12 Internalization (Confocal)

$$FRET(\%) = \left(\frac{I_1 - Io}{I_1}\right) x100$$

I_1 = Intensity after bleaching

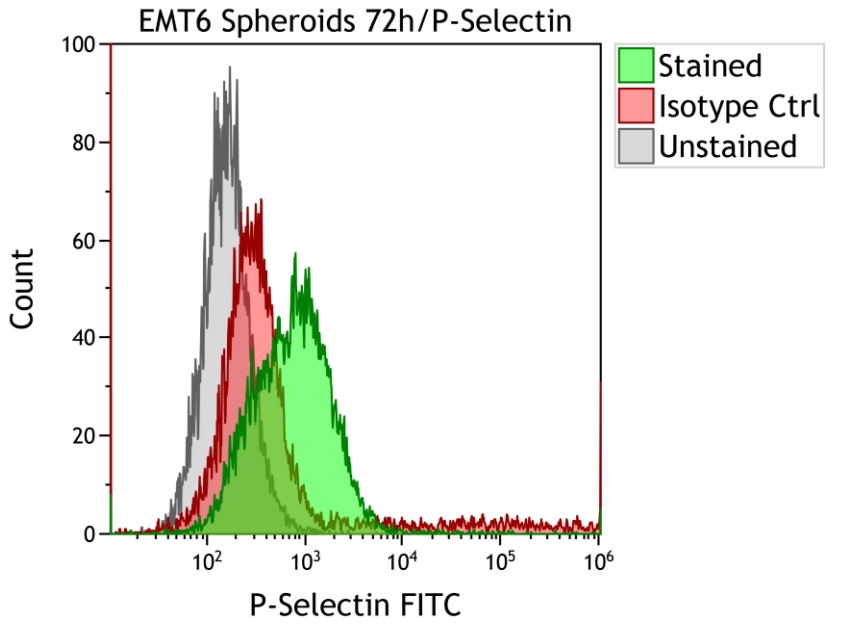
Io = Intensity before bleaching



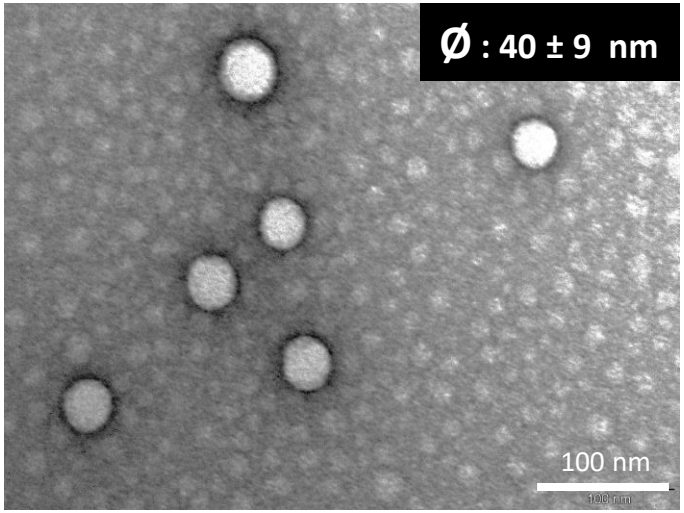
- The C6 Micelles seem to internalize faster than C12 micelles
- After 3 hours of Micelles incubation with EMT6 cells, the C12 micelles showed more FRET effect, probably due to their lower CMC values (3uM for the C12 vs. 4 uM for the C6)
- The Micelle-monomer equilibrium moves towards the monomer state during the interaction with the membrane, or micelles require longer internalization times than the monomers

Results obtained

P-Selectin Expression in EMT6 spheroids



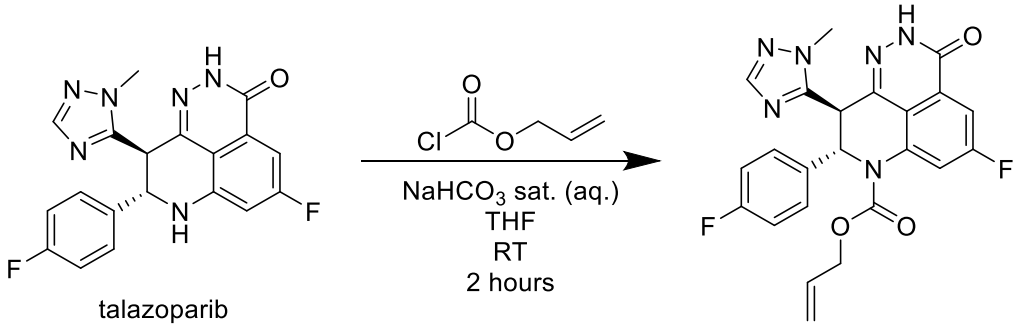
Marker	X-Med
All	779.54
All	311.18
All	160.59



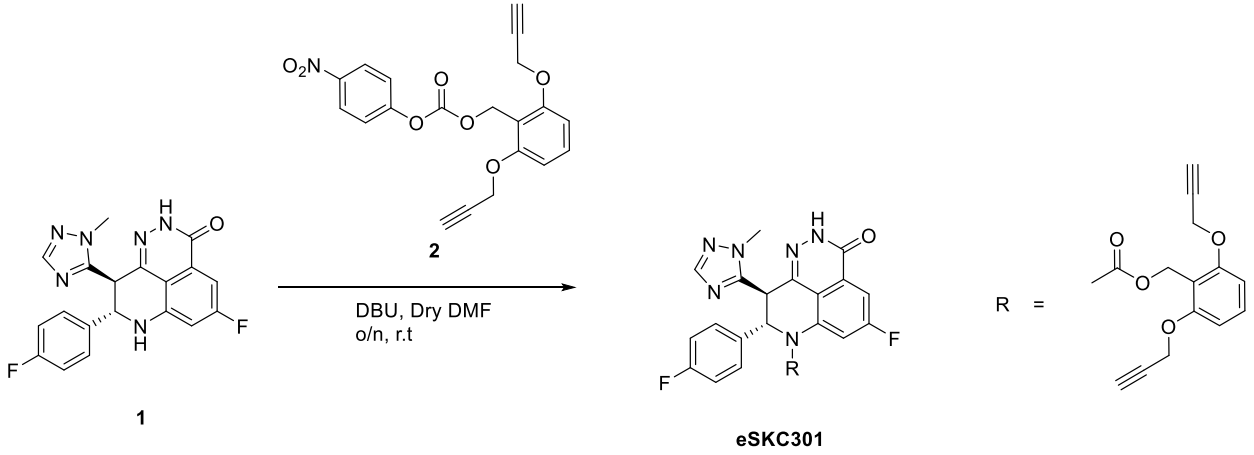
Results obtained

- Synthesis of Pro-Talazoparib

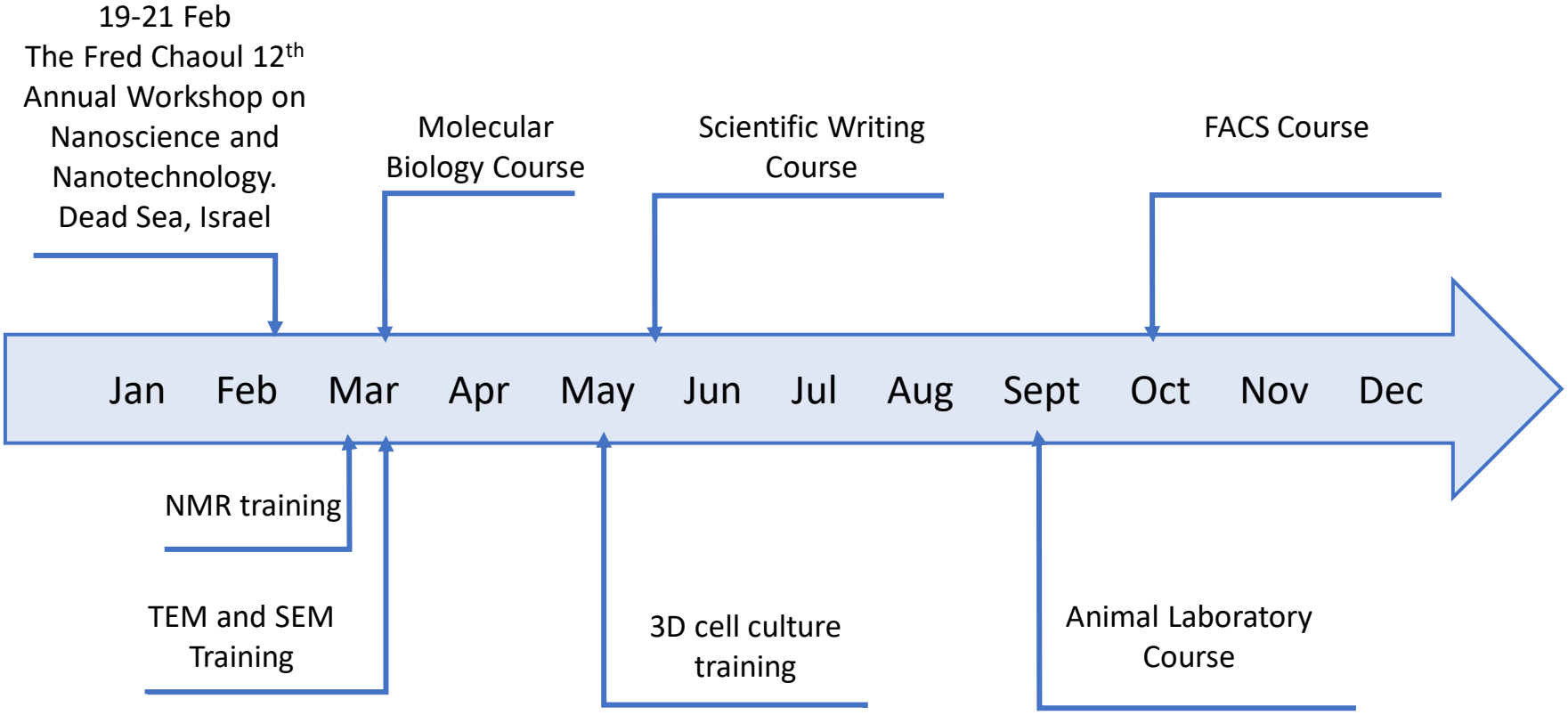
1)



2)



Local training

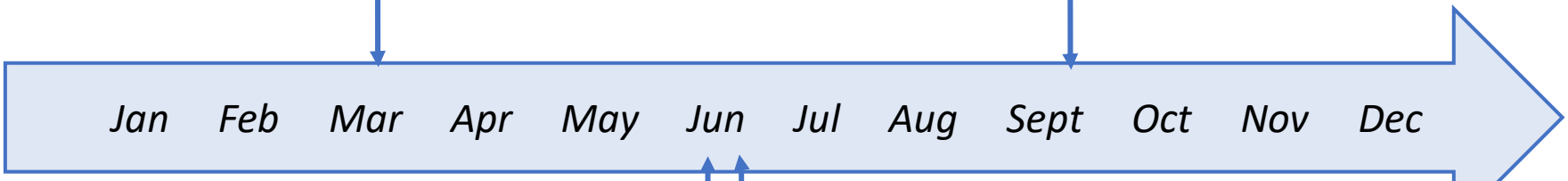


Communication and dissemination

Network-wide training 2019

25-29 March
1st Meeting and
Training Event.
Eindhoven,
Netherlands

23-27 Sep
2nd Training Event
Basel, Switzerland



PhD studies

- University: Tel Aviv University
- Title of PhD: Nanomedicine
- Enrolment: 9/12/2018

6-7 June
Bioorthogonal and
Bioresponsive
Edinburgh, UK

5 June
Mid-term Check
Edinburgh, UK