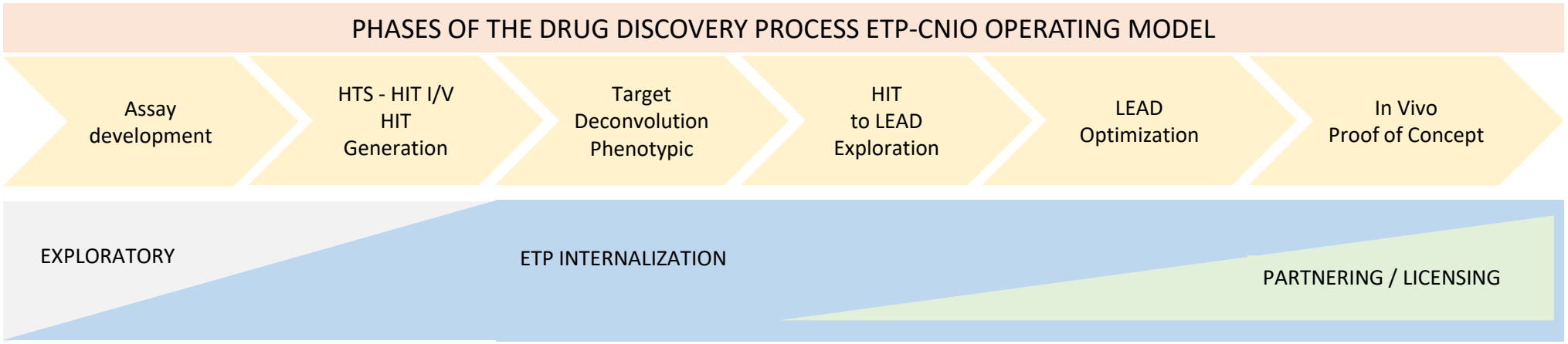
ETP-CNIO PIPELINE SUMMARY (non confidential)

July 2023

ETP-Pipeline 2023

cnio stop cancer



Exploratory Projects CNIO	*ETP Internalized Projects*	*Licensed Projects*
TDP2 inhibitors / Screening / Hit val. / F Cortes	CDK8 inhibitors / CNIO / PoC / Toxicology / Ready TT CDK8-is for NK cells activation / A. Perez (CNIO-HULP)	PIM inhibitors -> INFLECTION
PrimPol inhibitors / Screening / Hit Val. / J Mendez	MASTL inhibitors-PROTACs / M Malumbres / LO-HtL	IBL-100s Sublicense to Mysthera
MIDKINE / Screening / M.S. Soengas	TRF1 direct inhibitors / M Blasco / Hit Validation	Dual PIM-PI3K inhibitors -> INFLECTION
RET inhibitors / Screening / I Plaza	SETD8 inhibitors / O F Capetillo / HTS / Hit Generation	Triple PIM-PI3K-mTOR inhibitors -> AUM
Phenotypic Screening / Several Targets Mendez / Barbacid / Losada (Antitumorals Library)	RANK-is / E G Suarez ERC-PoC / HTS / Hit Validation	AUM-302 Orphan drug designation FDA
In Vivo PK studies: M Valiente / Capetillo / Peinado	NUDIX5 Inhibitors / R. Wright–CRG-UIC / Hit Val. / HtL	ATR inhibitors -> MERCK
Synthesis of Chemical Tools. Valiente / Capetillo / Djouer / Soengas / Blasco / Efeyan	Protein Degraders / C. Mayor -IRB / HTS / Hit Val.	PI3K inhibitors -> TOTUS Medicines
		FOXO Activators -> REFOXY

ETP-Pipeline 2023

ETP Internalized Projects
CDK8 inhibitors / CNIO / PoC / Toxicology / Ready TT CDK8-is for NK cells activation / A. Perez (CNIO-HULP)
MASTL inhibitors-PROTACs / M Malumbres / LO-HtL
TRF1 direct inhibitors / M Blasco / Hit Validation
SETD8 inhibitors / O F Capetillo / HTS / Hit Generation
NUDIX5 Inhibitors / R. Wright–CRG-UIC / Hit Val. / HtL
Protein Degraders / C. Mayor -IRB / HTS / Hit Val.

CNIO´s CDK8 Inhibitors

Highlights

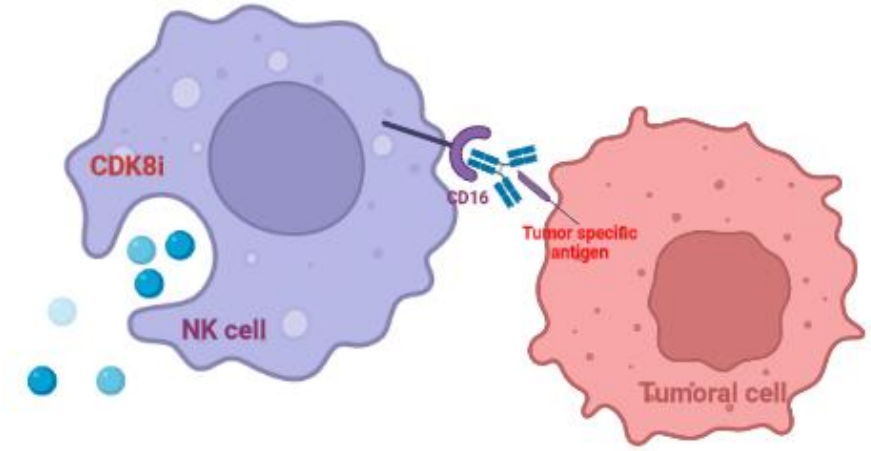
- ❑ A CDK8 inhibitor will presume a “First in Class” therapy to treat cancer.
- ❑ RVU120 (SEL120) Phase 1b in patients with AML or high risk myelodysplatic syndrome. A second phase 1 trial is recruiting to treat solid tumors.
- ❑ ETP has identified low-nM and picomolar selective CDK8-is (KinomeScan™) , in particular ETP-518.
- ❑ ETP-CDK8-is are cell active in the nM range in functional assays.
- ❑ Anti-proliferative data in +40 cell lines: Id. of sensitivity.
- ❑ ETP-518 is orally bioavailable
- ❑ PK-PD and efficacy study with ETP-518 with positive results.
- ❑ Chemical series with clear SAR (> 100 analogues). Patent application filed. WO2017033019
- ❑ No major toxicity observed in rats upon PO administration of 50 mg/kg QD during 14 days and zebrafish studies.
- ❑ Ready for Technology Transfer / partnering.

NEXT:

Preclinical characterization of ETP-518 as “Best in Class CDK8-i” (We need partnering for further development).

- ❑ Additional application / indication of CDK8-is: CDK8 involvement in NK activation (in collaboration with Antonio Pérez CNIO FIBHULP)

CNIO´s CDK8-is: NK Cells Activation (Collaboration with A. Pérez CNIO-FIBHULP)



ADCC therapy
Binding of mAbs to tumor-specific antigens
results in the activation of NK cells via the activation of the activating receptor CD16
CDK8- inhibition
Mediated STAT1–Ser727 non phosphorylation activates the cytolytic activity of NK cells

Preliminary Results

CDK8/19 inhibition (ETP-518) showed promising results in combination with anti-GD2 antibody. Increased the cytotoxicity of normal NK against neuroblastoma and sarcoma cell lines.

MASTL Inhibitors

HIGHLIGHTS. Collaboration with M. Malumbres CNIO.

- ❑ MASTL is a mitotic kinase that is involved in progression and resistance to therapy of tumors with high CIN (Prostate, Breast…).
- ❑ *First in class* project. Not MASTL-is in clinical trials or advanced stages, only poorly characterized inhibitors at discovery level.

- ❑ Lead Optimization Phase. SAR/SPR generated for MASTL activity and ADMET.
- ❑ ETP-53184 identified as initial lead MASTL inhibitor biochemical IC_{50} = 29.4 nM. BRET celular assay: ETP-184 active at nanomolar level.
- ❑ ETP-53184 good selectivity. KinomeScan (S(35)= 0.124; S(10)= 0.055).
- ❑ ETP-53184 good *in vitro* metabolic stability / CYP inhibition profile, but residual micromolar hERG binding (Patch Clamp ordered).
- ❑ In vivo PK ETP-184 showed oral bioavailability. Levels above the EC_{50} of its cell activity in plasma and peripheral compartments.

- ❑ ETP-715 as new lead with nanomolar biochemical / celular MASTL inhibition, good selectivity KinomeScan (S(35)= 0.139; S(10)= 0.052) and clean hERG.
- ❑ Exploration of ETP-715 has led to identification of a set of compounds with better overall properties and in vivo bioavailability after IP administration.
- ❑ However, 715's series showed suboptimal oral bioavailability (OB).
- ❑ Identification of the liability responsible for OB limitation -> Solubility-Stability in GIS fluids / Intestinal permeability / Efflux
- ❑ We have started the optimization of these molecules trying to modulate these aspects.

- ❑ IP space available current ETP-CNIO MASTL inhibitors.

- ❑ Exploration of chemically distinct back-up series has started to evaluate potential improvements of liabilities observed in ETP-715´s series.

NEXT: *In vitro/vivo* PoC studies with available MASTL-is in selected therapeutic settings.

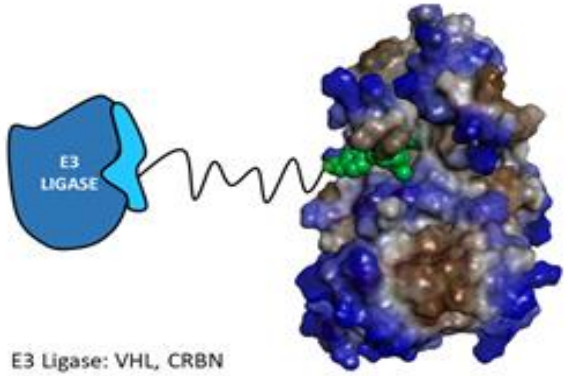
MASTL PROTACs

HIGHLIGHTS. Collaboration with M. Malumbres CNIO.

173 MASTL PROTACs synthesised so far

- ❑ Several PROTACs are currently in clinical trials, confirming the value of this strategy in DD

- ❑ Discovery of PROTACs for MASTL degradation as a complementary strategy to inhibition.
- ❑ *First in class* project. Not MASTL PROTACs reported.



- ❑ Design of PROTACs using proprietary MASTL ligand(s).
- ❑ Use of MASTL ligands solvent exposed area to install the linkers and E3 ligase ligands.
- ❑ A collection of > 150 PROTACs-like molecules has been synthesized.
- ❑ ETP-823 (and analogues) have been discovered as strong degraders of MASTL kinase.
- ❑ DC_{50} in the nanomolar range / Dmax ≈ 100%. MASTL depletion lasted up to 72h.
- ❑ PROTAC mode of action validated.
- ❑ Example: ETP-823 study by proteomics showed that MASTL was the most significantly depleted protein (6h / 24h treatments) out of 5565.
- ❑ Only other 5 proteins were affected significantly (6h / 24h treatments) although less than MASTL kinase.
- ❑ ETP-823 is bioavailable (IP administration) with levels above its DC_{50}.
- ❑ Other analogues improved the potency of ETP-823 and were also bioavailable.

NEXT

- ❑ Use selected PROTACs in PoC studies (cells) in selected therapeutic settings.
- ❑ To carry out PK/PD (degradation of MASTL) in *in vivo* xenograft mice models with selected PROTAC(s).
- ❑ Continuation of SAR/SPR exploration around ETP-823 towards the selection of advanced candidates with rigidified linkers (better ADMET properties).

Therapeutic settings for MASTL-is / PROTACs

Prediction of sensitivity of ETP-CNIO MASTL modulators in high CIN tumors (Geoff Macyntire CNIO did the analysis for Breast Cancer)
Collaboration with TAILOR-BIO (spin off company from GM research). Expand the prediction to other tumor types.

Article

A pan-cancer compendium of chromosomal instability

976 | Nature | Vol 606 | 30 June 2022

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Ruben M. Drews[1], Barbara Hernando[2], Maxime Tarabichi[3,4], Kerstin Haase[3,5], Tom Lesluyes[3], Philip S. Smith[1], Lena Morrill Gavarró[1], Dominique-Laurent Couturier[1,6], Lydia Liu[3,7], Michael Schneider[1], James D. Brenton[1,8,9], Peter Van Loo[3], Geoff Macintyre[1,2,10] & Florian Markowetz[1,10]

G. Macyntire

Potential biomarker for MASTL inhibitor response: CIN signature CX3 (from our Nature paper) is correlated with MASTL expression in breast cancers in TCGA (Figure 1) and across breast cancer cell lines (Figure 2).

CX3 represents copy number changes accumulated in the tumour due to impaired homologous recombination suggesting that inhibition of MASTL may be synthetic lethal with impaired HR.

We will test ETP-CNIO MASTL-is / PROTACs in recommended cell lines for validation…

Figure 1

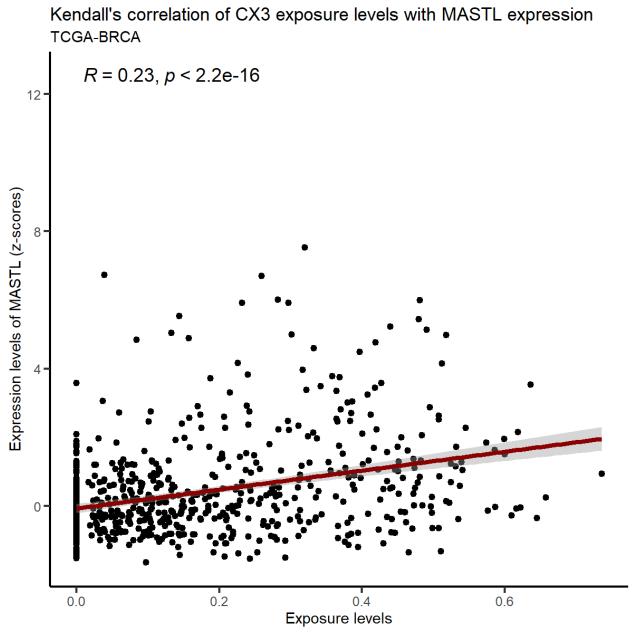
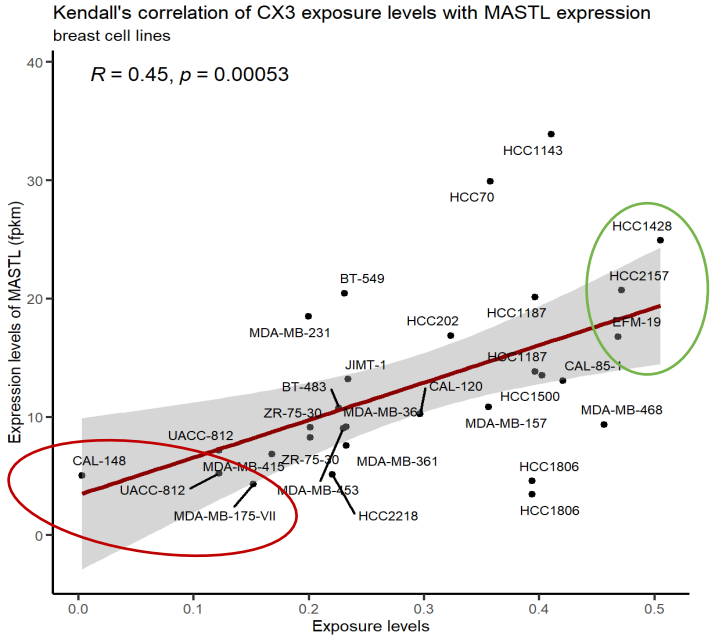


Figure 2



Therapeutic settings for MASTL-is / PROTACs

Mayo Clinic researchers have demonstrated the importance of MASTL-is in resistant Prostate cancer.
Collaboration with Mayo Clinic. Test ETP-CNIO MASTL-is/ PROTACs in their models.

Cell Reports Medicine

CellPress
OPEN ACCESS

Article

Harnessing transcriptionally driven chromosomal instability adaptation to target therapy-refractory lethal prostate cancer

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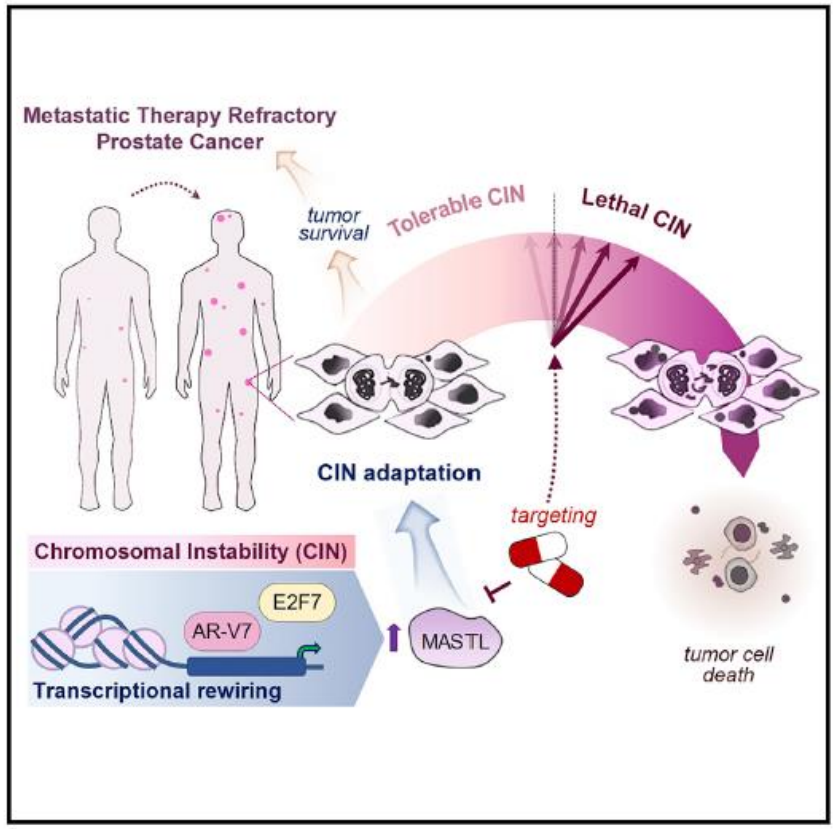

In brief

Dhital et al. unveil a CIN tolerance mechanism in metastatic therapy-resistant PCa involving *MASTL* upregulation by atypical transcription factors to restrain lethal chromosome defects and ensure tumor cell survival. Targeting CIN adaptation triggers tumor cell death in metastatic therapy-refractory PCa, increasing survival of pre-clinical models.

Therapeutic settings for MASTL-is / PROTACs

Combination of MASTL-is / PROTACs with DNA damaging agents / DNA Damage Response inhibitors / Radiotherapy

frontiers in Cell and Developmental Biology

MINI REVIEW
published: 23 November 2018
doi: 10.3389/fcell.2018.00162

The Oncogenic Functions of MASTL Kinase

*Kamila Marzec[1] and Andrew Burgess[1,2]**

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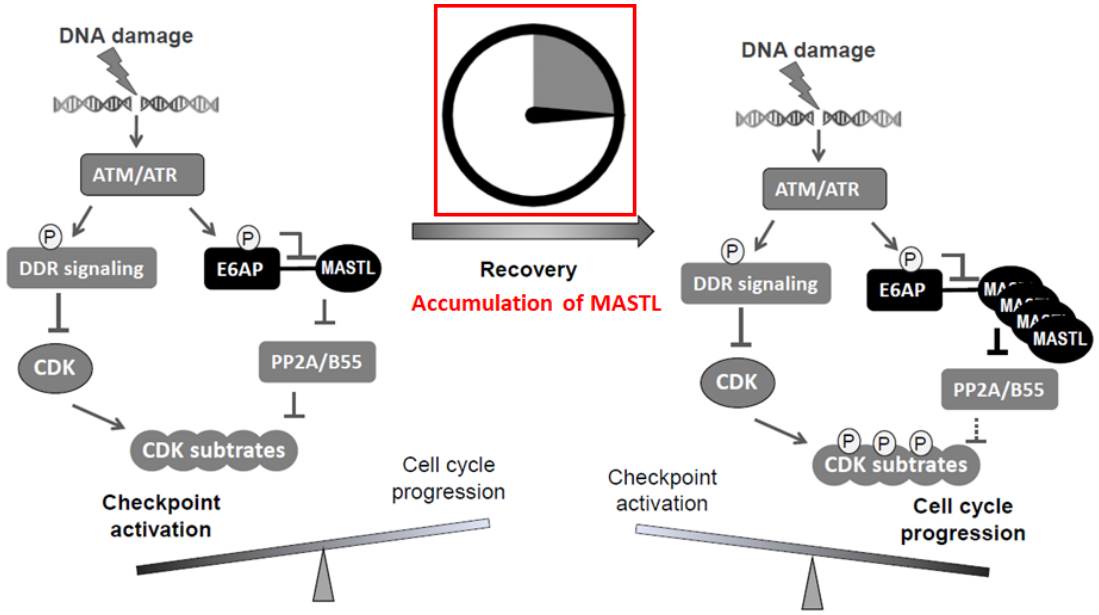
The second possibility for targeting MASTL is in combination with DNA damaging agents. The rationale for this is based on results showing that MASTL is critical for promoting checkpoint recovery from DNA damage (Peng et al., 2010, 2011; Wong et al., 2016). Consequently, overexpression of MASTL has been associated with resistance to cisplatin (Wang et al., 2014) by accelerating checkpoint recovery (Wong et al., 2016). Conversely, knockdown of MASTL can sensitize cancer cells to cisplatin, radiotherapy and 5-fluorouracil (5FU) in several cancer types (Wang et al., 2014; Nagel et al., 2015; Uppada et al., 2018; Yoon et al., 2018), most likely by preventing cells from re-starting the cell cycle following damage.



TRF1 Inhibitors

HIGHLIGHTS. Collaboration with M.A. Blasco CNIO.

- ❑ TRF1 is part of the Shelterin complex. Innovative validated target (MAB lab) for cancer therapy (First in Class).
- ❑ TRF1 depletion is compatible with mouse viability showing a good “therapeutic window”.

- ❑ TTG and ETP have collaborated in the last few years towards the discover of small molecules inhibitors of TRF1.
- ❑ We have identified several pathways and their inhibitors as indirect modulators of TRF1 binding at telomeres.
- ❑ The results are summarized in Nature Communications 2017 and EMBO Molecular Medicine 2019.

- ❑ The most specific and elegant approach to inhibit TRF1 would be the discovery of direct inhibitors.

- ❑ ETP has set up a proximity alpha assay to detect the binding of purified TRF1 to Tel-DNA.
- ❑ After screenings campaigns with ETP-Libraries (ca. 2.2K cpds) we have identified potential hit molecules that inhibit the binding of TRF1 to dsTel-DNA.
- ❑ The hits were counter-screened to avoid false positives by targeting dsTel-DNA and the assay read-out.

- ❑ Selected molecules were transferred to RYVU Therapeutics for follow up and further validation.
- ❑ RYVU carried out similar screenings to validate these potential hits.

- ❑ The conclusion of the whole data set was that compounds are able to bind hTRF1.
- ❑ It was proposed that the functionality of these compounds should be validated with EMSA experiments.

- ❑ ETP-CNIO has validated the functionality if ine representative hit by EMSA in dose response experiments. Estimated Kd: 66-110 μM.
- ❑ Hit to lead phase to optimize the potency of this hit series is underway.

SETD-8 Inhibitors

HIGHLIGHTS. Collaboration with O. Fernández-Capetillo and O. Llorca CNIO.

- ❑ SETD8 is the only lysine methyltransferase that can specifically monomethylate the histone H4 at Lys20.
- ❑ SETD8-mediated protein modifications are largely involved in the regulation of cell cycle, DNA repair, gene transcription, cell apoptosis, and other processes.
- ❑ The aberrant expression of SETD8 is closely linked to the proliferation, invasion, metastasis, and poor prognosis of a variety of cancers.

- ❑ *First in class* project. There are not SETD8-is in clinical trials or advanced stages.
- ❑ The project started from a potential SETD8 inhibitor found by OFC group with poor properties in terms of potency, selectivity, pharmacology and IP.
- ❑ OFC group has developed genetic models to validate the target and SETD8-inhibitors developed along the Project.

- ❑ ETP set up a biochemical assay with the catalytic domain of SETD8 (Commercial / OLL) and H4 peptide as substrate.
- ❑ Screening of ETP-800´s and ETP-Covalent Ligands libraries (1.3K) , and rationally proposed SAM mimic molecules led to Id. of quite a few micromolar hits.
- ❑ Some of these hits were validated with Full Length SETD8 (OLL) and H4 peptide as substrate.
- ❑ Two hits were also active using reconstituted Nucleosomes (OLL) as substrate with FL SETD8.

- ❑ ETP has set up an alternative assay that gave enough window to measure H4K20Me1 inhibition in cells in 6h.
- ❑ OFC lab has set up a High Throughput Microscopy assay based on these precedents. It will be used to identify H4K20Me1 inhibitors (i.e. SETD8-is).

NEXT

- ❑ SAR of current hits
- ❑ HTM cell based screening of alternative ETP-Libraries to identify H4K20Me1 inhibitors (i.e. SETD8-is).