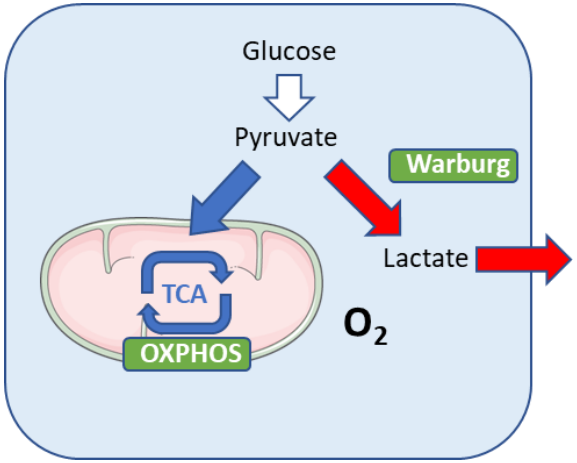
Novel OXPHOS and DHODH inhibitors

July 2019

Background

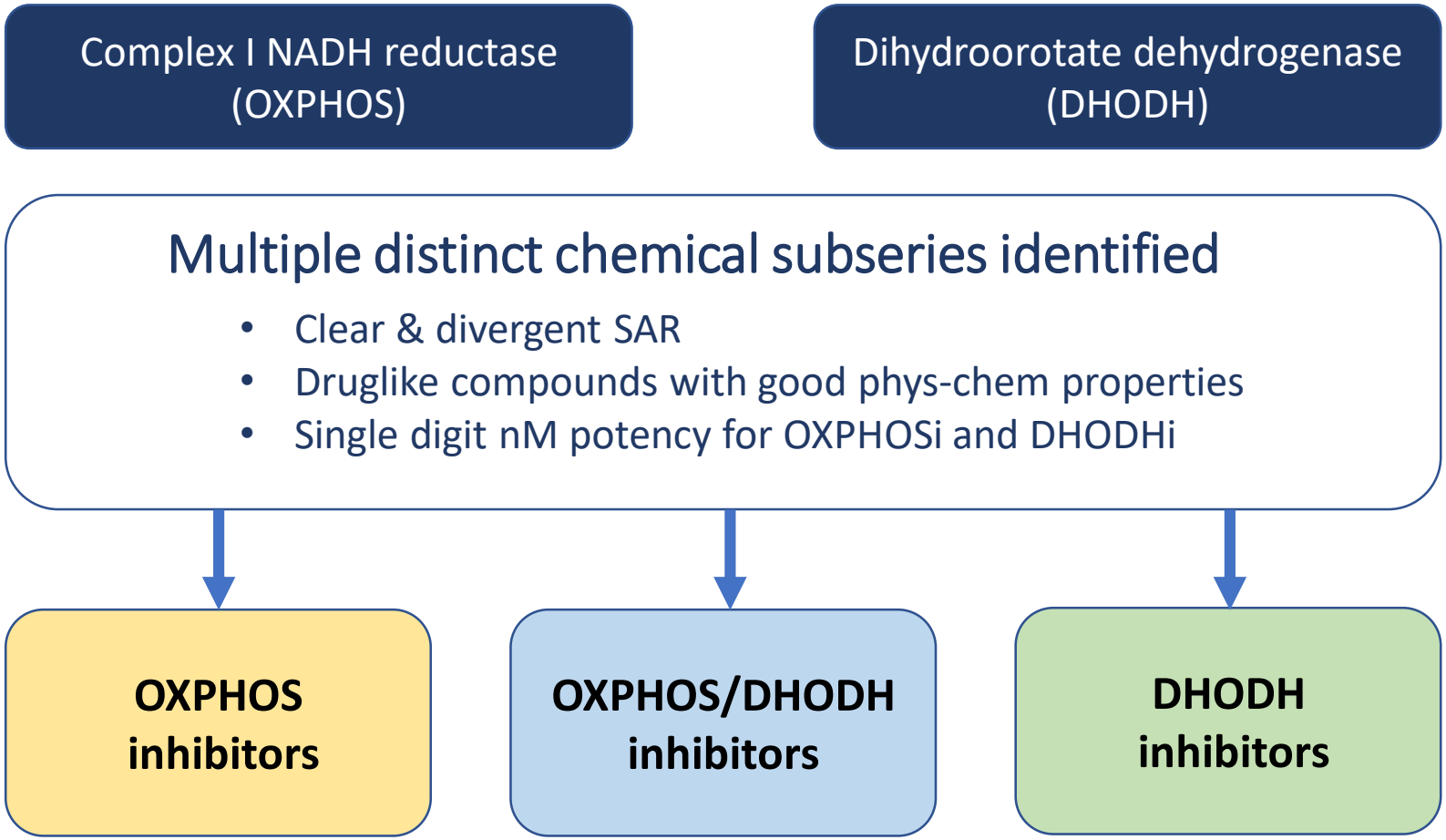
- Partnership with prof Olivier Feron (UCLouvain)
  - KOL in tumor metabolism: utilization of lactate as a key carbon source in tumors (Corbet & Feron, Nature Reviews Cancer 2017)

- Phenotypic drug discovery approach to identify selective inhibitors of mitochondrial – but not glycolytic - cancer cell metabolism.



Background

- Target deconvolution revealed 2 distinct target:



OXPHOS and DHODH inhibition: divergent SAR

	EC50 (µM)	OXPHOS		DHODH*	
	ID	HeLa (oxphos)	HeLa (glycolysis)	MOLM13	MOLM13 (+Uridine)
OXPHOS inhib	CIM057118	0.002	>30	>30	>30
	CIM118379	0.010	>1	2.3	4.7
	CIM057875	0.016	>1	6.5	6
	CIM117534	0.017	>30	5.6	4.5
OXPHOS/DHODH inhib	CIM137846	0.013	5	0.005	0.7
	CIM056996	0.034	>10	0.026	0.9
	CIM136603	0.063	>30	0.020	0.8
	Brequinar	5.6	4.5	0.017	>30
DHODH inhib	CIM136191	11	>30	0.001	5
	CIM136608	4	>30	0.001	3
	CIM136192	16	>20	0.006	4
	CIM136200	16	>30	0.006	6

*MOLM13 = highly DHODH-sensitive AML cell line

Background

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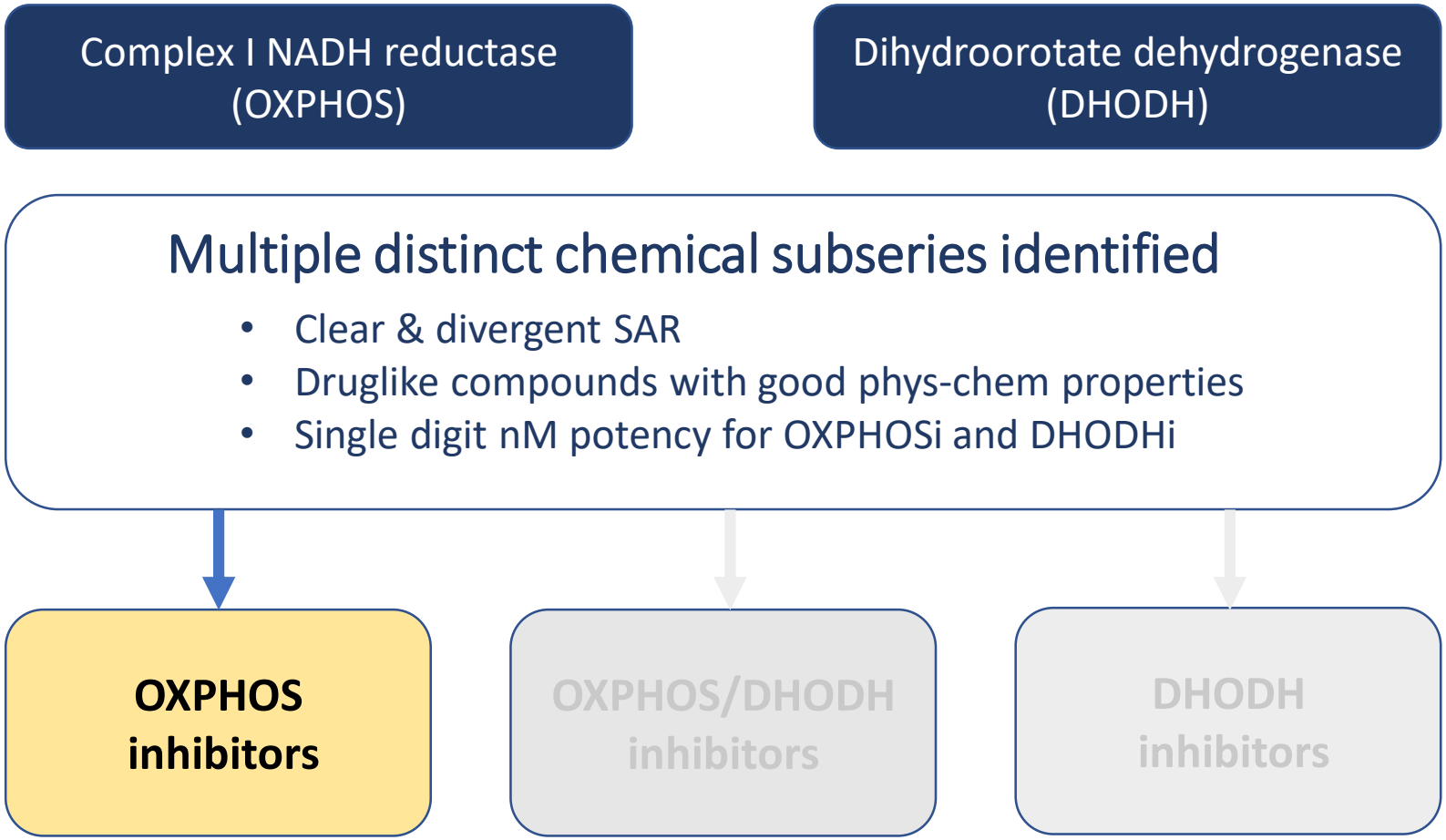
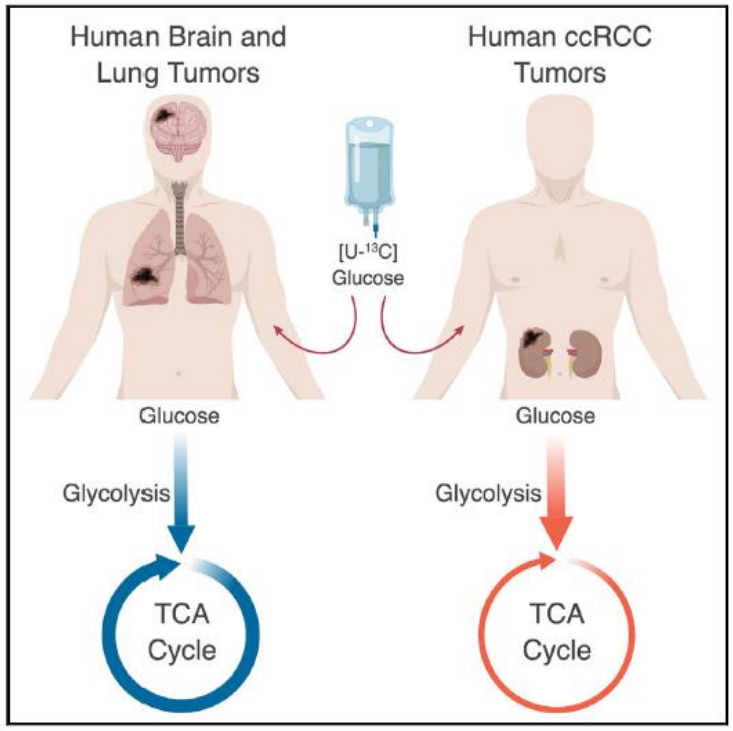


OXPHOS-dependent cancer subsets



- A **subpopulation of glioblastomas** with deletion of Enolase 1 are glycolysis-deficient and dependent on OXPHOS
- Many **neuroblastomas** are deficient in glycolysis, eg deficiency in phosphoglycerate dehydrogenase (PGD)
- **Mutations in the SWI/SNF complex in lung cancer** enhance OXPHOS and create a selective vulnerability to OXPHOS inhibition
- **KRAS-driven lung tumors** in mice are dependent on pyruvate carboxylase and pyruvate dehydrogenase
- **PTEN-null (but not PTEN WT) advanced prostate cancers** are dependent on OXPHOS
- **Melanoma brain metastasis** are characterized by increased reliance on OXPHOS
- **Chemoresistant AML** is dependent on OXPHOS for survival

Genetic hard-wiring of OXPHOS metabolic dependency in cancer cells

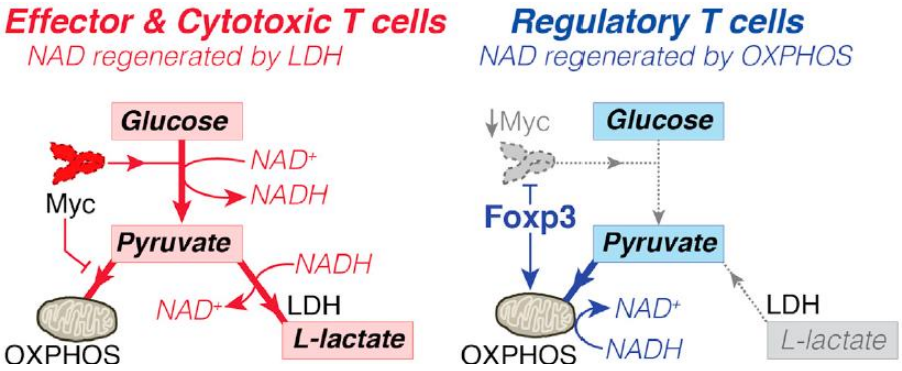
↓

Hypersensity to complex I inhibition

Molina et al., Nat Med 2018; Deribe et al., Nat Med 2018; Davidson et al., Cell Metab. 2016; Fischer et al., Cancer Discov 2019; Lee et al., Nature 2019; Naguib et al., Cell Reports 2018; Farge et al., Cancer Discov. 2017

Inhibition of OXPHOS mitochondrial metabolism enhances anti-tumor immunity

- **OXPHOS** mitochondrial metabolism contributes to an immune-suppressed pro-tumorigenic environment, favoring **T_{reg}s, MDSCs, M2 macrophages**.
- Anti-tumor **cytotoxic T-cells and M1 macrophages** require **glycolysis**



- Tumor cell oxidative metabolism is a barrier to PD-1 blockade in melanoma

Angelin et al., 2017 Cell Metab; Scharping et al., Canc Immunol Res 2017; Najjar et al., JCI Insight 2019.

Therapeutic hypothesis

- OXPHOS inhibitors are efficacious in OXPHOS-dependent tumor subsets, based on a dual mechanism:

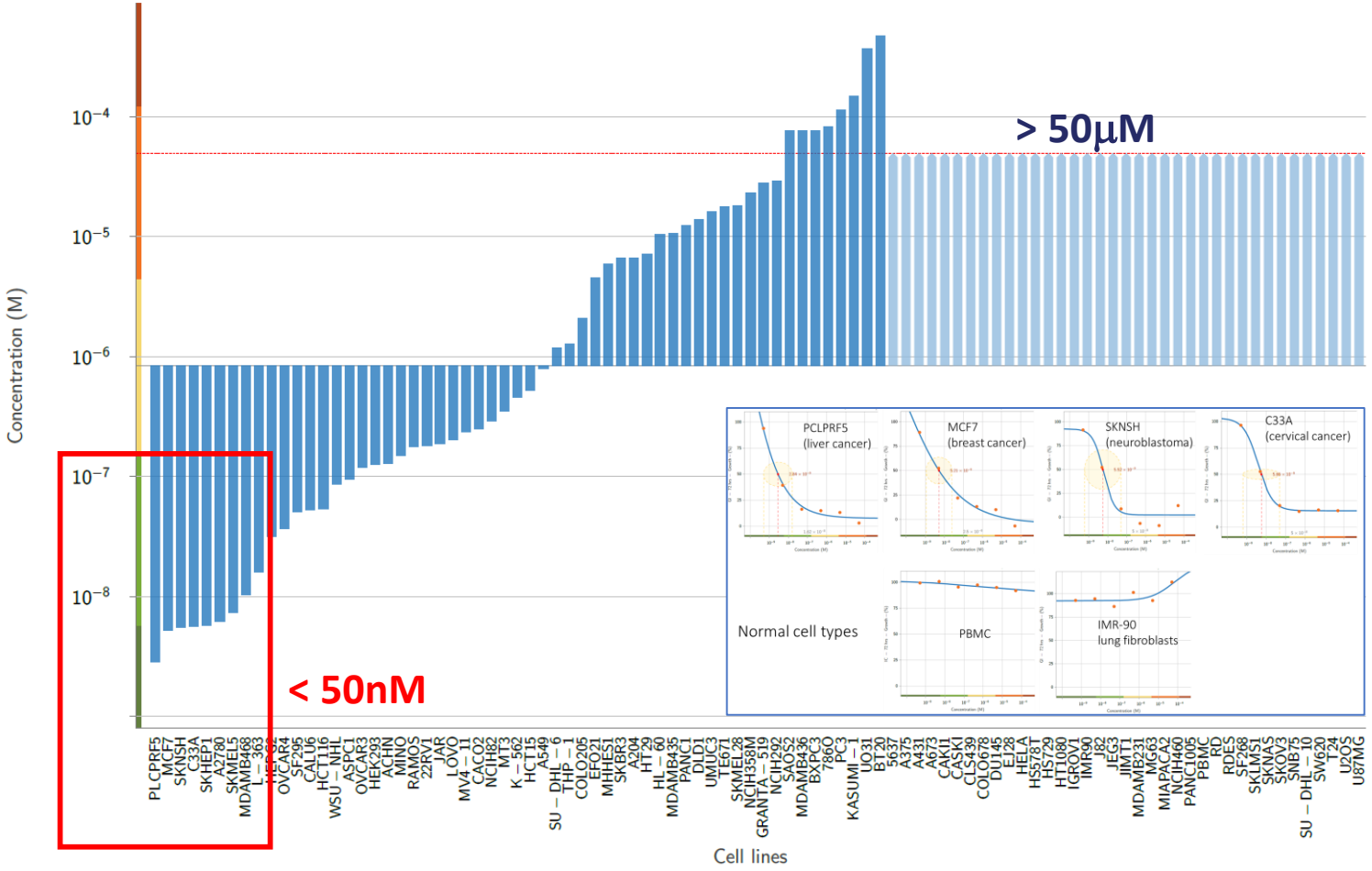
  1. **Genetic and environmental OXPHOS metabolic dependency** in cancer cells results in **hyper-sensitivity to complex I inhibition**

  2. OXPHOS inhibition **enhances anti-tumor immunity: cytotoxic** & **effector T-cells** in the tumor-microenvironment do not require OXPHOS whereas immune-suppressive **regulatory T-cells** do.

- Selective complex I inhibitors should have a robust therapeutic index in OXPHOS-dependent cancer types

OXPHOS inhibitors: cancer cell line sensitivity

- Sensitivity across cancer cell line panel reveals **hyper-sensitive human cancer cell lines <u>under standard culture conditions</u>, with 10 000x selectivity window** (insensitive cell lines, incl. PBMCs, fibroblasts)

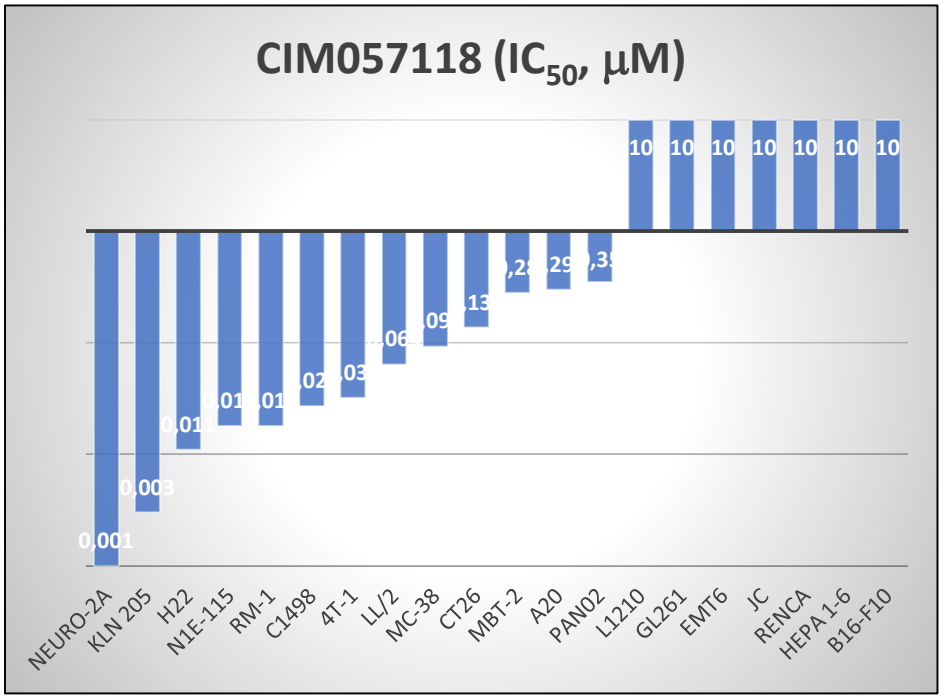


CIM057118

Lact/Glc 0.002/>30 µM

OXPHOS inhibitors: cancer cell line sensitivity

- Sensitivity across cancer cell line panel reveals **hyper-sensitive mouse cancer cell lines under standard culture conditions**

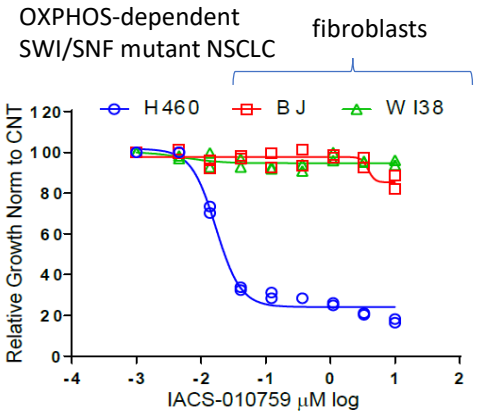


		CIM057118 (IC50, µM)
Neuroblastoma	Neuro-2a	0,001
Lung Cancer	KLN 205	0,003
Liver Cancer	H22	0,011
Neuroblastoma	N1E-115	0,018
Prostate Cancer	RM-1	0,018
Leukemia	C1498	0,027
Breast Cancer	4T-1	0,032
Lung Cancer	LL/2	0,064
Colorectal Cancer	MC-38	0,093
Colorectal Cancer	CT26	0,137
Bladder Carcinoma	MBT-2	0,28
Lymphoma	A20	0,299
Pancreatic Cancer	Pan02	0,35
Leukemia	L1210	10
Neuroblastoma	GL261	10
Breast Cancer	EMT6	10
Breast Cancer	JC	10
Renal adenocarcinoma	Renca	10
Liver Cancer	Hepa 1-6	10
Melanoma	B16-F10	10

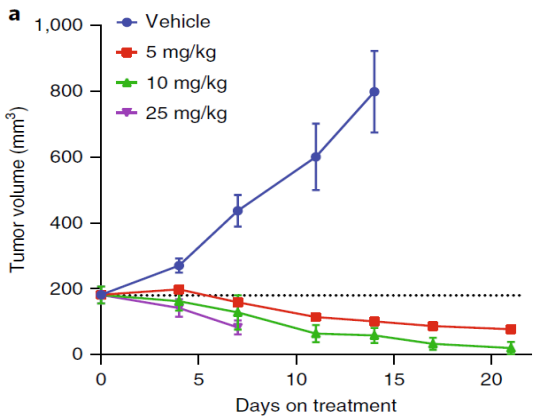
➢ Several mouse cancer models are OXPHOS-dependent and can serve as syngeneic (immune-competent) *in vivo* models

OXPHOS: competitive landscape

- IACS-010759 (MD Anderson Cancer Center) in two Phase I studies.
  - Novel, selective inhibitor of the MT-ND1 complex I subunit encoding NADH reductase
  - Funded by 'Moonshot' program
  - In phase I in relapse/refractory (R/R) AML, recruiting
  - In phase I in solid tumors and lymphoma, recruiting; expansion cohorts in Triple-negative Breast cancer (TNBC) and pancreatic ductal adenocarcinoma (PDAC).
  - Recently demonstrated to overcome resistance in PDTX ibrutinib-resistant B cell lymphoma mouse model



NB-1 xenograft model



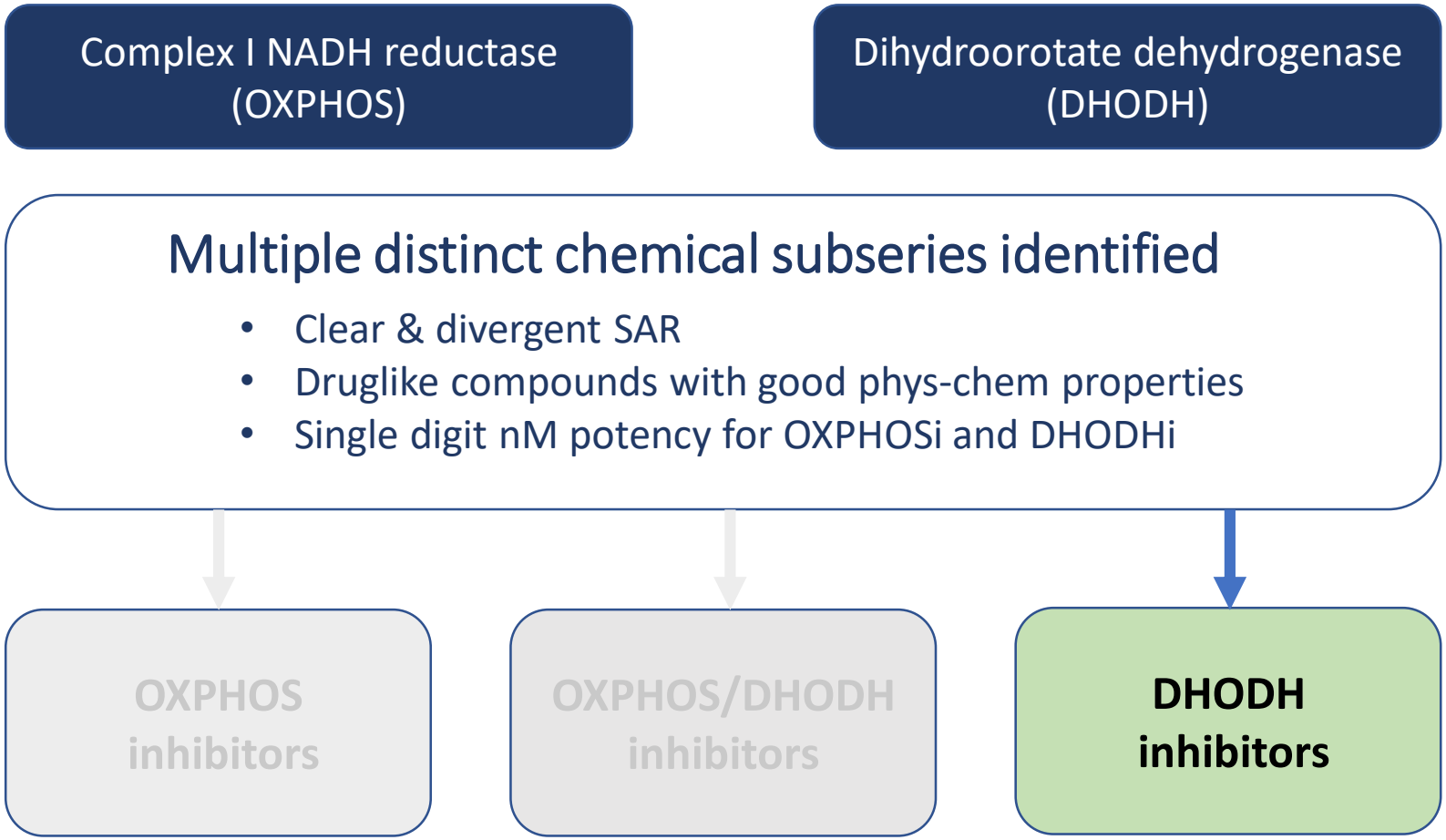
- Immunomet has developed biguanides on the basis of immunomodulatory effect of OXPHOS inhibition.
- A few academic research groups have recently published on other mitochondrial complex I inhibitors (tool compounds/early stage of development).

OXPHOS inhibitors: conclusions

- Potent and selective proprietary complex I NADH reductase inhibitors
- Several OXPHOS hyper-sensitive cancer subsets validated
- Immune-competent OXPHOS-dependent syngeneic mouse tumor models identified
- Lead optimization in progress to enable *in vivo* PoC
  - Lead series A
    - Single digit nM potency
    - Metabolic stability being optimized
  - Back-up series B
    - Potency optimization on-going (best current potency = 20 nM)
    - Promising preliminary ADME data

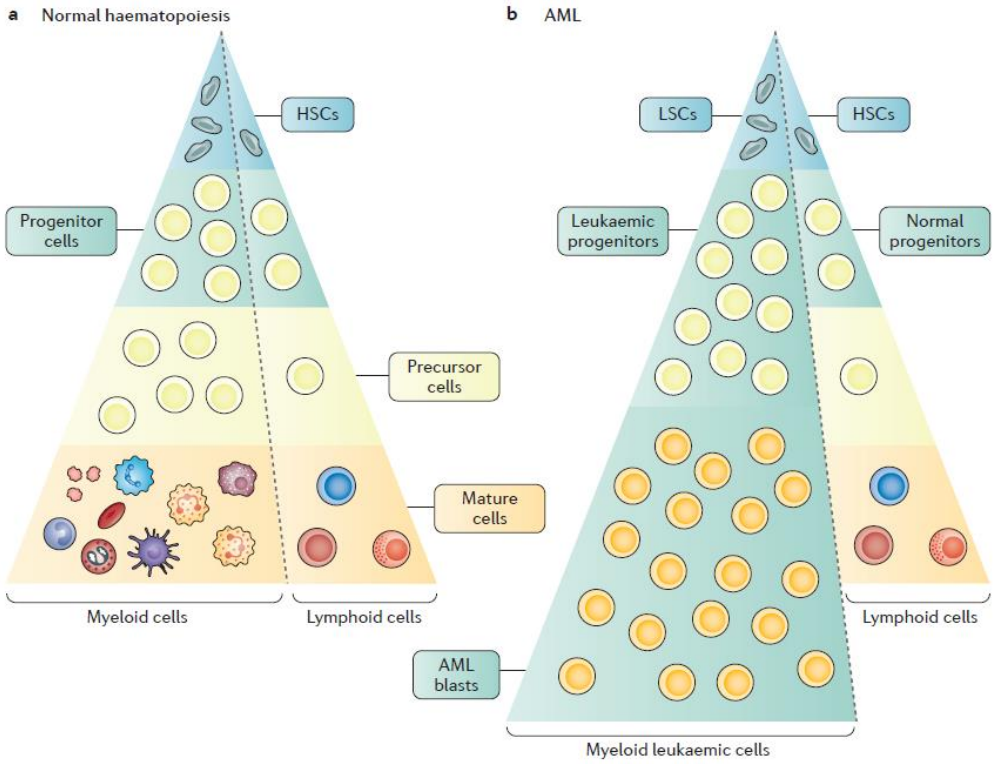
Background

- Target deconvolution revealed 2 distinct target:



Strong Rational in AML

- Acute myeloid leukemia: malignancy of **undifferentiated myeloid cells** in the bone marrow and in the periphery.
- Deadliest leukemia in western world: **21 000 new cases/year in US (10 000 deaths)**, survival at 5 years: 25%.
- Standard treatment is chemotherapy: Ara-c (cytarabine), daunorubucin. High response rate, but rapid relapse and poor prognosis (25% survival at 5 years) (associated with bone-marrow residing leukemic cells).
- Induction of AML differentiation with ATRA is a curative strategy in a small subset of AML (APL, 10 %)
- Novel treatments (approved 2017) focus on small genetic subsets (Flt3 mutant, IDH1 mutant).

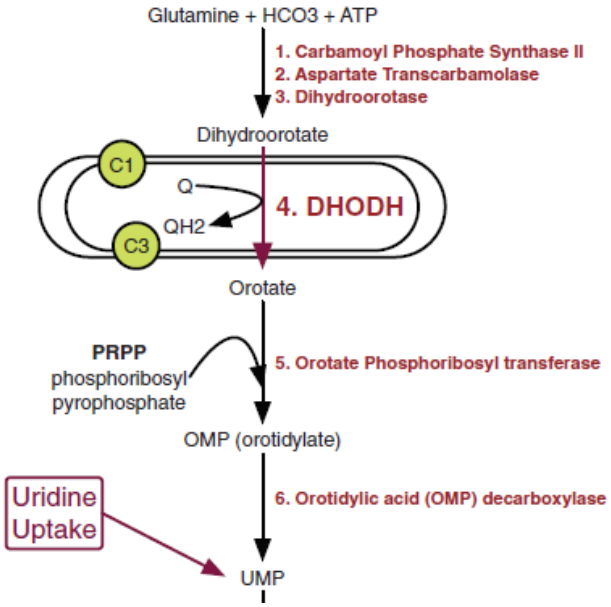


DHODH inhibition to treat AML

- **Inhibition or knock-down of DHODH**, an enzyme essential for *de novo* pyrimidine biosynthesis, **overcomes AML differentiation blockade, regardless of genetic subtype.**

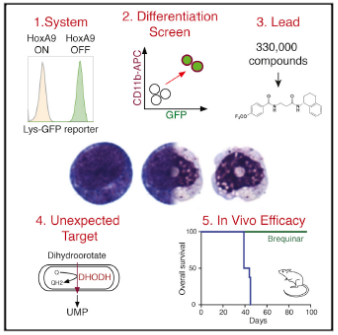
- **Validated using novel-generation DHODH inhibitors** (PTC299, BAY2402234, AG-636). AML proliferation highly sensitive to DHODHi, can be rescued by exogenous uridine (Sykes et al., Cell 2016; Cao et al., Mol Canc Ther. 2018; AACR 2018)

- Downstream inhibitors of DNA or RNA synthesis, or DNA damaging agents do not affect differentiation. Mechanism not yet fully understood



Inhibition of Dihydroorotate Dehydrogenase Overcomes Differentiation Blockade in Acute Myeloid Leukemia

Graphical Abstract



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In Brief

Inhibition of a metabolic enzyme involved in pyrimidine biosynthesis induces differentiation of leukemic cells, identifying a potential therapeutic approach for treating a range of acute myeloid leukemias, independent of their oncogenic driver.

Highlights

- A phenotypic differentiation screen identifies DHODH as a target in AML
- DHODH is a key link between pyrimidine synthesis and myeloid differentiation
- DHODH represents a metabolic vulnerability across a range of AML subtypes
- DHODH inhibitors show therapeutic potential in preclinical AML models.

DHODH inhibition: opportunities in solid tumors

- Several solid tumor indications have been proposed to be sensitive to DHODH inhibition:
  - DHODH inhibitors selectively inhibit the growth of **KRAS mutant tumors** (Koundinya et al., Cell Chem Biol. 2018)
  - Increased dependency on de novo pyrimidine biosynthesis in tumors with **loss of PTEN** (Brown et al., Mathur et al., Canc Discov. 2017)
  - DHODH inhibition increases **P53 synthesis and synergizes with nutlins** (P53 stabilization) --Ladds et al., Nature Comm 2018.

DHODH inhibition: overview

EC50 (µM)	OXPHOS		DHODH	
ID	HeLa (oxphos)	HeLa (glycolysis)	MOLM13	MOLM13 (+Uridine)
Brequinar	>30	>30	0.015	> 5
Teriflunomide	>30	ND	1.9	> 20
CIM136191	11	>30	0.001	5
CIM136608	4	>30	0.001	3
CIM136601	4	>30	0.002	3
CIM136192	16	>20	0.006	4
CIM133309	8	>30	0.010	2
CIM133311	>30	>30	0.042	2
CIM138403	4	25	0.004	2.3
CIM154449	8	>30	0.006	4.5
CIM138408	8	>30	0.007	2.1
CIM134112	>30	>30	<0.009	1.5
CIM137908	1.5	>30	0.050	11

*MOLM13 = highly DHODH-sensitive AML cell line (DHODH deficiency can be rescued by exogenous uridine)

- **Highly potent DHODH inhibitors available**

Conclusions and next steps

1. **Complex I NADH reductase** inhibitors:
    - Single digit nM potency
    - Selective impact on OXPHOS-dependent cancer subsets (glioblastoma, neuroblastoma, lung cancer, …)

2. **DHODH** inhibitors:
    - Single digit nM potency
    - Selective impact on AML. Potential opportunities in other myeloid malignancies (MDS), and other hematologic cancers (lymphoma's).

3. Unique dual **OXPHOS/DHODH** inhibitors identified
    - Potential in chemo-resistant AML.

- IP available on multiple compound sub-series.
- Lead optimization ongoing
- Timeline: *in vivo* PoC 2019, Preclinical candidate 2020

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