TRANSLATIONAL ONCOLOGY SUMMIT 2020

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Fast-tracking C010DS-Zn:

PARP1-agonist targeting cancer and M2-like macrophages

xylonix.io

24 Sept 2020, 10PM (UTC+8)

Jinhyuk Fred Chung, PhD

CSO @ Xylonix

Overview

- M2-like macrophages (**M2M**) shown to drive the core pathologies of **cancer** and **COVID19**
  - Cancer metastasis
  - T cell suppression leading to immune escape
  - Hyperprogression phenomenon post PD-1/PD-L1 treatment
  - Severe forms of COVID19 and Multisystemic Inflammation Syndrome in Children (MSI-C) post COVID19 recovery

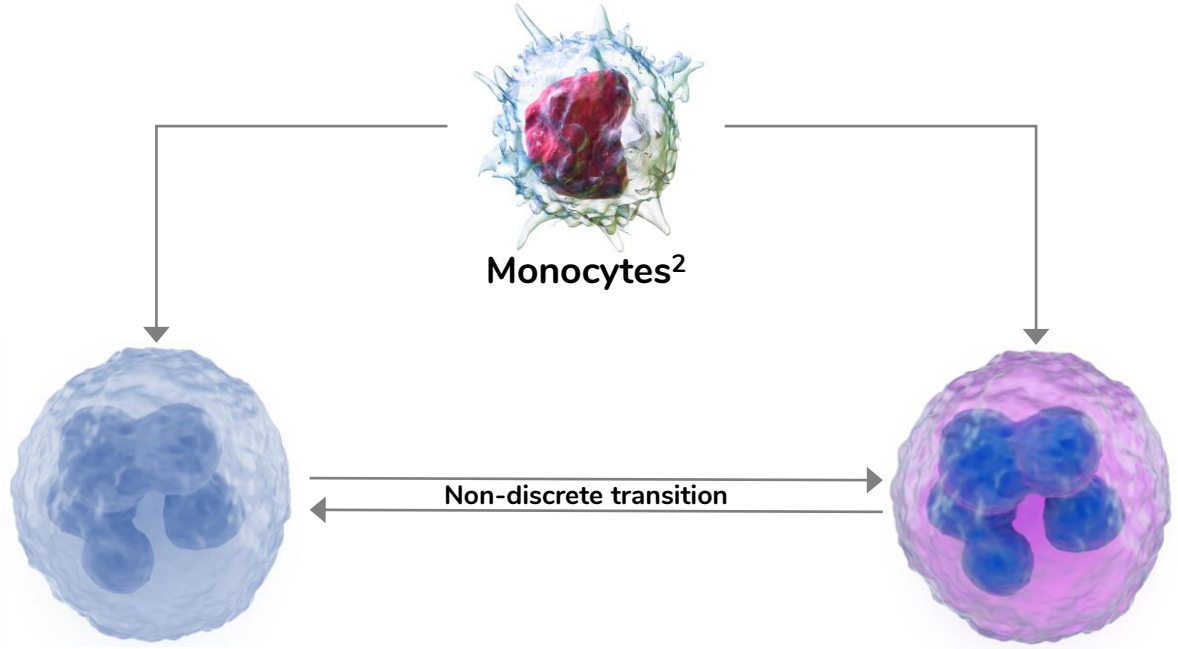
- Rationale-designed C010DS-Zn is a folate-ligand guided PARP agonist for triggering parthanatos in the following:
  - General solid cancers (via overexpressed FOLR-α and FOLR-β)
  - General blood cancers (via overexpressed FOLR-β)
  - M2-like macrophages and Tumor Infiltrating Macrophages (via overexpressed FOLR-β)

- Parthanatos is a programmed necrosis from hyperactived DNA repair response by PARPs with the advantages of:
  - High immunogenicity for robust immune response initiation
  - Precision targeting potential against wide cancer types with high mutation burden or mutagenic oncogenes such as mTP53

- Fast-track development of this previously unexplored anti-cancer mechanism by C010DS-Zn is being driven by:
  - High throughput ex-vivo screening against NGS-sequenced patient derived tumors and bioinformatic analysis for rapid identification of precision indication targets, further parthanatos mechanisms, and potential drug resistance mechanisms
  - In vivo tests using humanized mice for direct combination studies with pembrolizumab and other immunotherapeutic agents

Targeting M2-like macrophages (M2M) for treating cancer and COVID19

Defining M2-like macrophages[1]



M1 macrophage

Generally pro-inflammatory
Generally anti-tumor

- Phagocytic
- Produces TNFα, IL1β, **IL6**, IL12, NO, etc
- Acts as APC to CD4 T cell
- Stimulates CD8 T cell
- Direct tumor cell killing via NO
- Produces TNFα, IL1β, IL12, NO, etc

M2-like macrophages (M2a, M2b, M2c, M2d, etc)

Generally anti-inflammatory
Generally pro-tumor

- Non-phagocytic
- Produces TGFβ, **IL6**, IL10, EGF, VEGF, cathepsins, etc
- Main component of Tumor Associated Macrophages (TAM)
- Stimulates Treg
- Inhibits CD8 T cell
- Promotes metastasis and angiogenesis

1. Review: Funes et al (2018. Immunology. https://doi.org/10.1111/imm.12910
2. Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". *WikiJournal of Medicine* **1** (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436.

M2M as the key target in treating cancer and COVID19

Cancer metastasis

HEPATOLOGY AASLD

ORIGINAL

M2 macrophage-derived exosomes facilitate hepatocarcinoma metastasis by transferring $\alpha_M\beta_2$ integrin to tumor cells

Jindao Wu, Wen Gao, Qiyun Tang, Yue Yu, Wei You, Zhengshan Wu, Ye Fan, Long Zhang, Chen Wu, Guoyong Han, Xueliang Zuo, Yao Zhang, Zhiqiang Chen, Wenzhou Ding ... See all authors

First published: 28 June 2020 | https://doi.org/10.1002/hep.31432

Tumor Biology and Immunology — Cancer Research

M2 Macrophage-Derived Exosomes Promote Cell Migration and Invasion in Colon Cancer

Jingqin Lan[1], Li Sun[2], Feng Xu[1], Lu Liu[1], Fuqing Hu[1], Da Song[1], Zhenlin Hou[1], Wei Wu[1], Xuelai Luo[1], Jing Wang[3], Xianglin Yuan[2], Junbo Hu[1], and Guihua Wang[1]

Article | Open Access | Published: 16 May 2019

M2 macrophages promote NSCLC metastasis by upregulating CRYAB

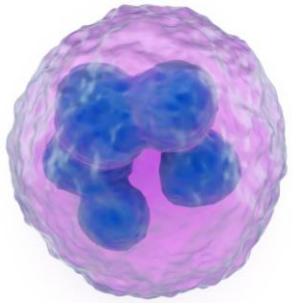
Zhe Guo, Jing Song, Junxia Hao, Hui Zhao, Xiaohui Du, Encheng Li, Yanbin Kuang, Fuquan Yang, Wei Wang, Jiong Deng & Qi Wang

Cell Death & Disease **10**, Article number: 377 (2019) | Cite this article

J Hematol Oncol. 2017; 10: 36. PMCID: PMC5286803
Published online 2017 Feb 1. doi: 10.1186/s13045-017-0408-0 PMID: 28143526

Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis via M2 macrophage-secreted CHI3L1 protein

Yulei Chen,[1] Siyuan Zhang,[1] Qizhi Wang,[2] and Xiaobo Zhang[1]



M2-like macrophages

A common enemy in our fight against CANCER & COVID19

Hyperprogression post immunotherapy

Translational Cancer Mechanisms and Therapy — Clinical Cancer Research

Antibody–Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade

Giuseppe Lo Russo[1], Massimo Moro[2], Michele Sommariva[3], Valeria Cancila[4], Mattia Boeri[2],

Trends in Cancer — CellPress REVIEWS

Opinion

Hyperprogression and Immunotherapy: Fact, Fiction, or Alternative Fact?

Jacob J. Adashek,[1] Ishwaria M. Subbiah,[2] Ignacio Matos,[3] Elena Garralda,[3] Arjun K. Menta,[4] Dhakshina Moorthy Ganeshan,[2] and Vivek Subbiah[2,*]

Severe COVID19 and MSI-C post COVID19

Anti-SARS-CoV-2 IgG from severely ill COVID-19 patients promotes macrophage hyper-inflammatory responses

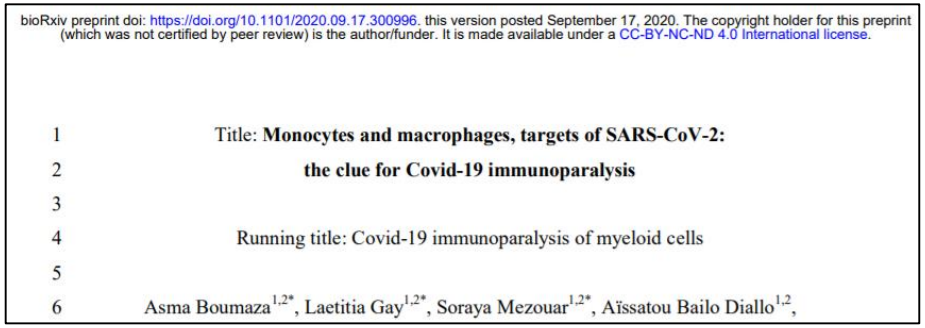
Willianne Hoepel[1,2†], Hung-Jen Chen[3†], Sona Allahverdiyeva[1,2,4], Xue Manz[5], Jurjan Aman[5],

1 Title: **Monocytes and macrophages, targets of SARS-CoV-2:**
2 **the clue for Covid-19 immunoparalysis**
3
4 Running title: Covid-19 immunoparalysis of myeloid cells
5
6 Asma Boumaza[1,2*], Laetitia Gay[1,2*], Soraya Mezouar[1,2*], Aïssatou Bailo Diallo[1,2],

Latest discoveries on the role of M2M involvement in COVID19

Anti-SARS-CoV-2 IgG from severely ill COVID-19 patients promotes macrophage hyper-inflammatory responses

Willianne Hoepel[1,2†], Hung-Jen Chen[3†], Sona Allahverdiyeva[1,2,4], Xue Manz[5], Jurjan Aman[5],



Title: **Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis**

Running title: Covid-19 immunoparalysis of myeloid cells

Asma Boumaza[1,2*], Laetitia Gay[1,2*], Soraya Mezouar[1,2*], Aïssatou Bailo Diallo[1,2],

Key discoveries

- COVID19 directly infects all monocytes and macrophages via "**abortive infection**", **reprogramming them toward M2M without any cytopathic effects** (Bounmaza et al., 17 Sept 2020)
- This reprogramming led to **massive tissue infiltration by the M2M**, and overproduction of **IL6**, **IL10**, and **TGFβ** – the hallmark pathologies of severe COVID19
- An independent research further showed that the hyper-inflammatory responses in severe COVID19 cases were produced by **M2M, stimulated by afucosylated anti-COVID19 IgG** (Hoepei et al., 13 July 2020).
- Heopei et al. also showed that the cytokines released by the anti-COVID19 IgG stimulated M2M caused **microvascular thrombosis** and **endothelial damages**, providing the pathological explanations for the **autoimmune thrombosis** and the Multisystemic Inflammation in Children (**MSI-C**) post COVID19 recovery.
- This "two-component" pathology of COVID19 may pose a major hidden obstacle in current vaccine development efforts

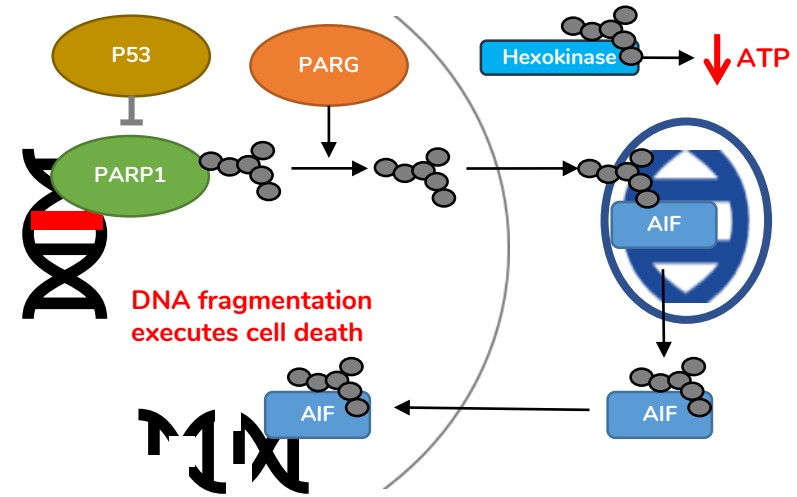
Selective elimination of the COVID19-reprogrammed M2M is a promising approach for treating severe COVID19, stopping its long-term health effects, and safe use of the vaccines

C010DS-Zn: First-in-Class parthanatos inducer against cancer and M2M

Parthanatos and Xylonix approach

- Parthanatos is a programmed necrosis from PARP-hyperactivation that results in immunogenic cell death
- Parthanatos machinery is independent and orthogonal to that of apoptosis, reducing cross-resistance risks
- PARP-hyperactivation is a DNA-repair response, making cell types with **higher mutation burden** or **mutagenic oncogenes more vulnerable**.
- Xylonix has developed several **folate-guided PARP agonist compounds** that amplify Zn^{2+} pharmacology toward selective parthanatos induction in the target cells, from **C005D-Zn** to **C010DS-Zn with varying potency**
- Zn^{2+} pharmacology was chosen for its simultaneous inhibition of apoptotic caspases[1] in protecting PARP1 toward parthanatos

Parthanatos at a glance



www.impactjournals.com/oncotarget/ Oncotarget, 2018, Vol. 9, (No. 4), pp: 4485-4495

Research Paper

Assessment of folate receptor alpha and beta expression in selection of lung and pancreatic cancer patients for receptor targeted therapies

Jiayin Shen[1], Yingwen Hu[1,2], Karson S. Putt[2], Sunil Singhal[3], Haiyong Han[4], Daniel W. Visscher[5], Linda M. Murphy[6] and Philip S. Low[1,2]

Published OnlineFirst December 1, 2009; DOI: 10.1158/0008-5472.CAN-09-2050
Published Online First on December 1, 2009 as 10.1158/0008-5472.CAN-09-2050

Immunology

Folate Receptor β Is Expressed by Tumor-Associated Macrophages and Constitutes a Marker for M2 Anti-inflammatory/ Regulatory Macrophages

Amaya Puig-Kröger,[1,2] Elena Sierra-Filardi,[1] Angeles Domínguez-Soto,[1] Rafael Samaniego,[3]

Table 2

Main features of apoptosis, necrosis and parthanatos.

	Apoptosis	Necrosis	Parthanatos
Plasma Membrane	Blebbing	Swelling and lysis	Lysis but no blebbing
ANV/PI assay	+/−	+/+	+/+
TUNEL assay	+	−	+
Caspase-3 activation	+	−	+ (not mandatory)
PARP dependence	−	−	+
AIF	Translocation to the nucleus (not always)	No translocation	Translocation to the nucleus

SOURCE: Soriano et al. (2017) DOI: 10.1038/srep41340

1. Eron et al., (**2018**) *ACS Chem Biol.* DOI:10.1021/scschembio.8b00064

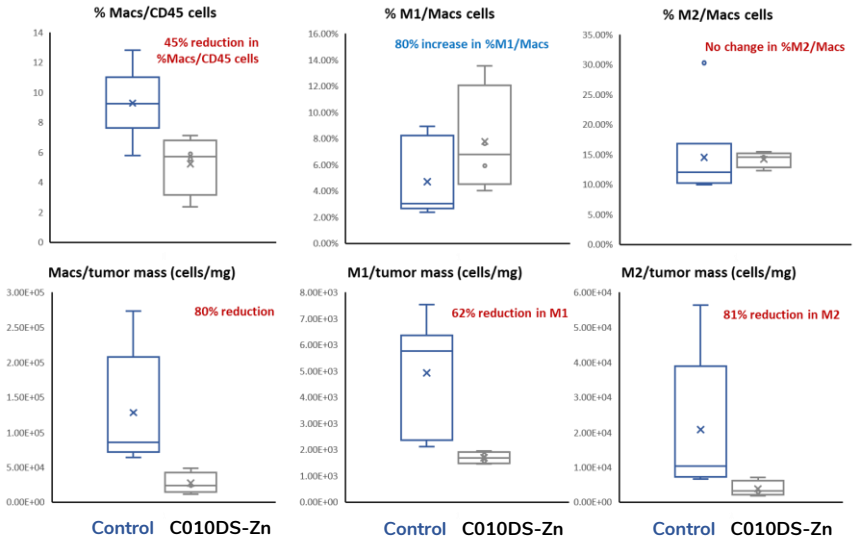
C010DS-Zn takes the center stage post COVID19

Xylonix parthanatos agents at a glance

	C005D-Zn	C008D-Zn	C010DS-Zn
In vitro anti-cancer IC50 (24h)[1]	290 μM Zn^{2+}	90 μM Zn^{2+}	16 μM Zn^{2+}
In vitro anti-cancer IC50 (48h)[1]	63 μM Zn^{2+}	44 μM Zn^{2+}	0.7 μM Zn^{2+}
In vitro anti-cancer IC95 (48h)[1]	537 μM Zn^{2+}	100 μM Zn^{2+}	1.6 μM Zn^{2+}
In vivo anti-M2M effects	-	+	+++
In vivo toxicity observed (acute/repeat)	-/-	-/-	-/-

[2]Basal serum Zn^{2+} level in healthy individuals is about 150 μM

M2M reduction in tumor by C010DS-Zn[3]



Xylonix response to the pandemic –fast track C010DS-Zn

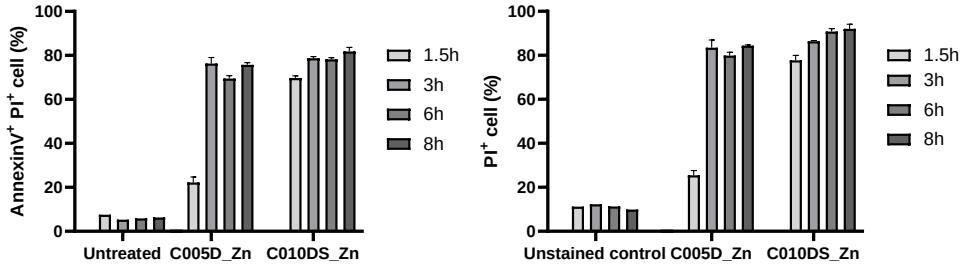
- Prior to the pandemic, C005D-Zn development was prioritized for its excellent safety profiles
- The pandemic brought forth an emergent need for an effective anti-M2M agent, lifting the potential regulatory concerns for C010DS-Zn's capacity to reduce M2M
- **Given the clinical and regulatory bottleneck for new first-in-human studies, Xylonix decided to fast-track C010DS-Zn for COVID19 application, with subsequent repurposing back to oncology use.**

1. In vitro murine cancer cell line tests performed at **Invitrocue** and at **Champions Oncology**, cell type undisclosed for future publication
2. Vale et al., (**2014**) *Eur J Clin Nut*. https://doi.org/10.1038/ejcn.2013.250
3. In vivo murine cancer subQ xenograft model, with an undisclosed cell type and dosing amounts for future publication. The in vivo dosing did not show direct anti-tumor effect

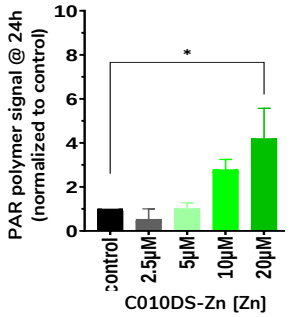
Breakthrough advantages of Xylonix parthanatos agents against cancer

Rapid target parthanatos in hours[1]

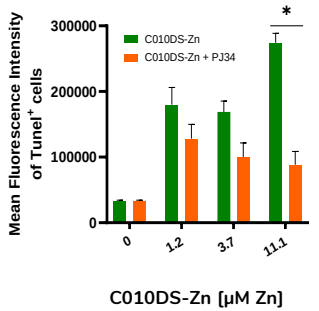
In vitro kinetics of a cancer cell line parthanatos by C005D-Zn or C010DS-Zn @ 4X IC50



PARP agonism by C010DS-Zn

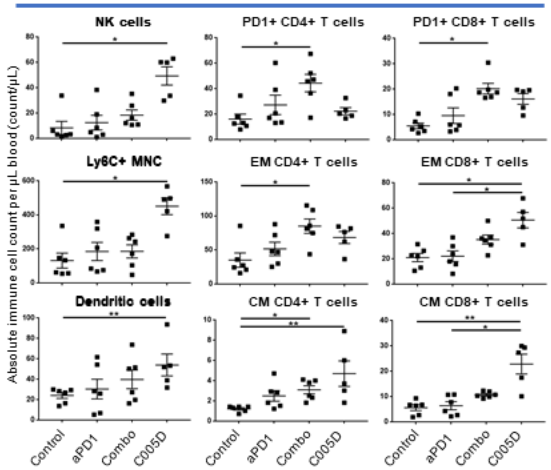


TUNEL signal intensity



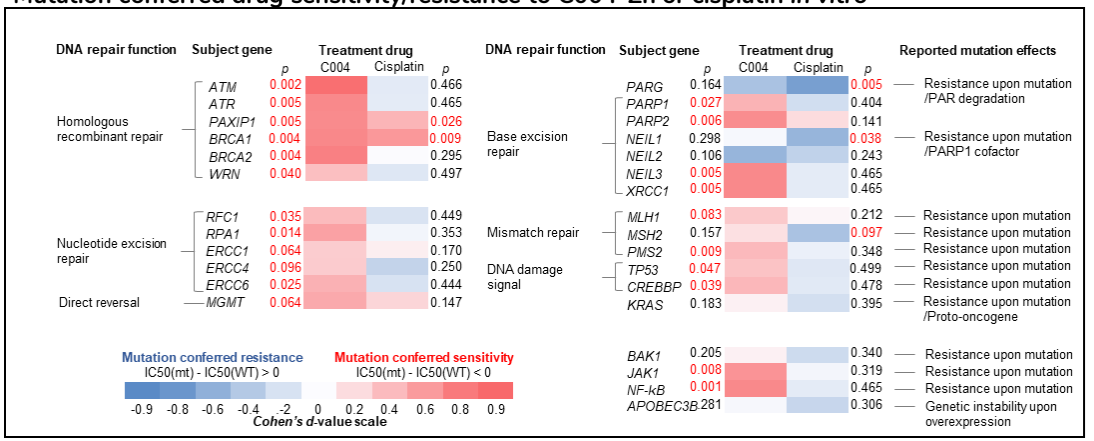
Immune response initiation potential[2]

Peripheral blood leukocytes in CT26 bearing Balb-c mice



Broad precision indication potential[3]

Mutation conferred drug sensitivity/resistance to C004-Zn or cisplatin *in vitro*



1. In vitro murine cancer cell line tests performed at **Invitrocue.** Cell type undisclosed for future publication. 4X IC50 concentration applied in the kinetic study.
2. In vivo murine cancer subQ xenograft model using CT26 on Balb-c , performed at **IMCB - A*Star**
3. Analysis based on the 50 human cancer cell line screening at **Crown Biosciences** using C004-Zn

Overcoming the challenges of fast-tracking a First-in-Class

Breakthrough advantages of Xylonix parthanatos agents against cancer

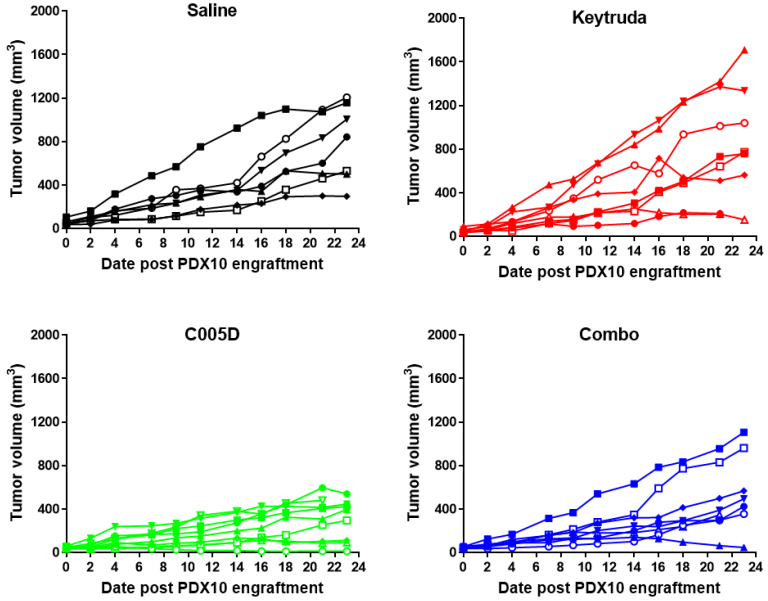
"Gaining a clear understanding of how a drug works before it enters clinical trials is the intelligent route to drug discovery and could increase the likelihood for drug success."

Mechanism matters. Nat Med 16, 347 (2010). https://doi.org/10.1038/nm0410-347

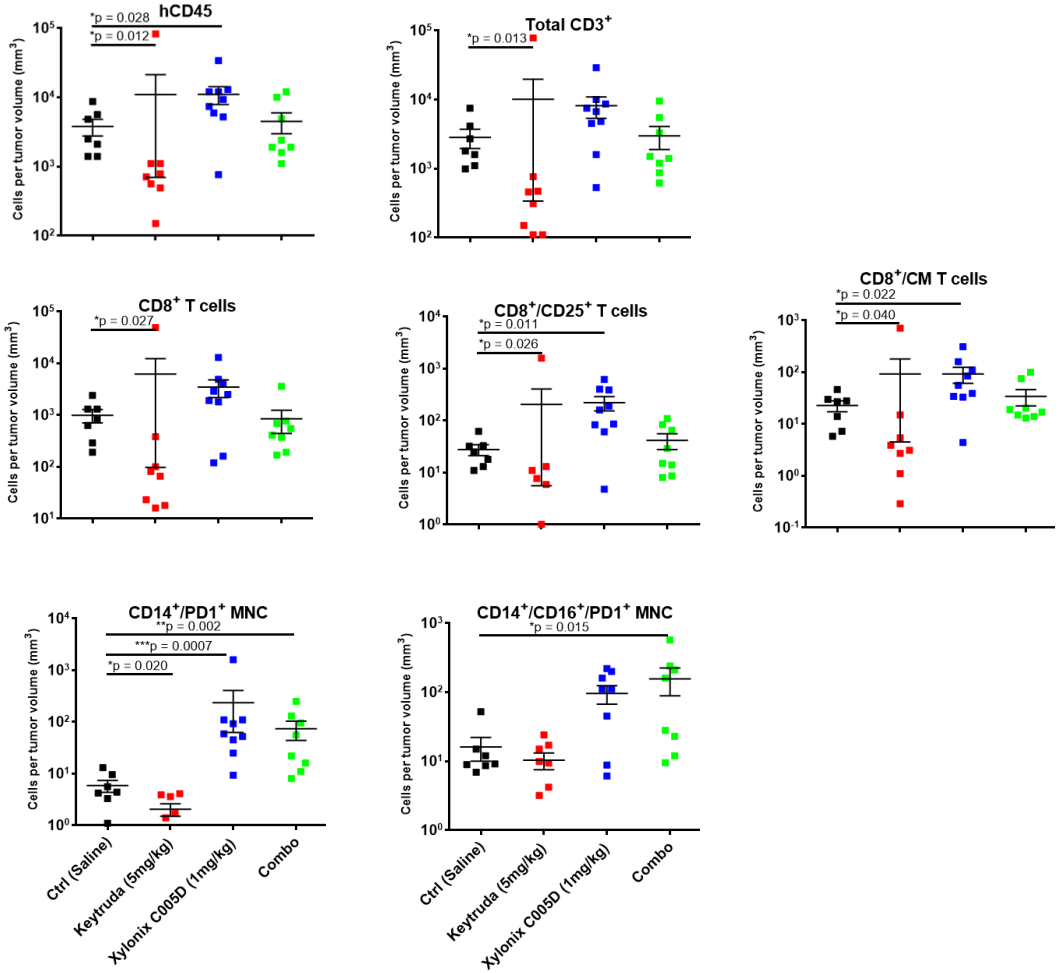
- First-in-Class drug development comes with unique challenges, especially with C010DS-Zn
  - The very mechanism of parthanatos toward the cell death execution is still new & unclear
  - No available literatures on the pharmacological benefits or side effects of using parthanatos against cancer or otherwise
  - Its wide-spectrum indication potential is overwhelming – how much of it can be translated? And how do we define it?
  - Its secondary immunogenic anti-cancer mechanism adds further dimensions in consideration of future study designs, especially when considering combination studies with aPD-1 agents.
- Our answers to the hurdles
  - Aggressive use of ex *vivo* patient derived tumor (PDX) screening platform with bioinformatic analysis
  - Continuous translation: PDX *ex vivo* -> PDX *in vivo* (immunodeficient) -> PDX *in vivo* (humanized) validation
  - Utilization of humanized mouse *in vivo* tests for the immune response and mechanism validation

Importance of *in vivo* validation on humanized immunity: a case of C005D-Zn

Tumor growth kinetics of HCC on HuMice



Characterization of tumor infiltrating immunity

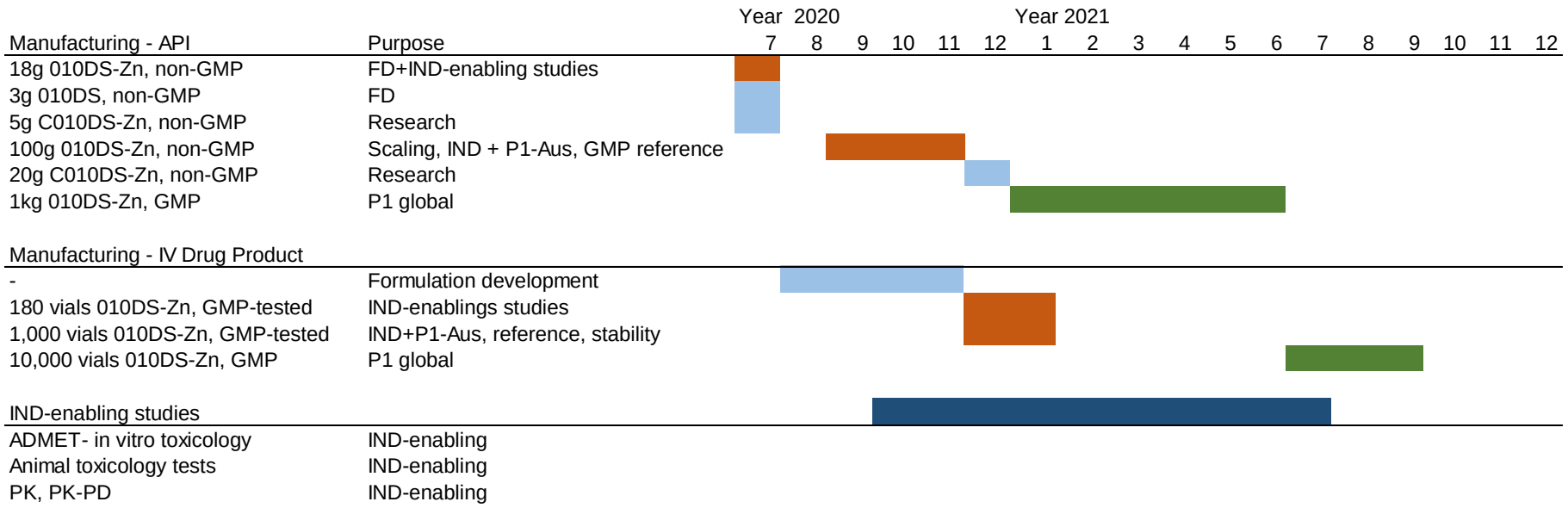


A direct combination study using humanized mice between C005D-Zn and pembrolizumab revealed deleterious effects of macrophage immune response initiation when using pembrolizumab

- Previous syngenic studies using murine aPD1 did not display an anti-synergy in combination use
- It was later published by other groups that the IgG4(S228P) platform used by leading aPD1 agents was responsible for M2M-mediated reversal of the aPD1 into an immune suppressive agent via FcR interactions[1]
- This became the basis of our focus on combination studies with pexidartinib and C010DS-Zn development

1. Lo Russo et al., (**2019**) Clin Cancer Res. doi: 10.1158/1078-0432.CCR-18-1390

Fast track development progress on C010DS-Zn



Manufacturing - API	Purpose
18g 010DS-Zn, non-GMP	FD+IND-enabling studies
3g 010DS, non-GMP	FD
5g C010DS-Zn, non-GMP	Research
100g 010DS-Zn, non-GMP	Scaling, IND + P1-Aus, GMP reference
20g C010DS-Zn, non-GMP	Research
1kg 010DS-Zn, GMP	P1 global
Manufacturing - IV Drug Product	
-	Formulation development
180 vials 010DS-Zn, GMP-tested	IND-enablings studies
1,000 vials 010DS-Zn, GMP-tested	IND+P1-Aus, reference, stability
10,000 vials 010DS-Zn, GMP	P1 global
IND-enabling studies	
ADMET- in vitro toxicology	IND-enabling
Animal toxicology tests	IND-enabling
PK, PK-PD	IND-enabling

- So far we have been on-schedule since the start of the C010DS-Zn fast-track initiative
  - Early efforts mainly focused on CMC for the fastest availability of GLP/GMP supply
  - In view of the pandemic, we schedule to prepare sufficient quantities of GMP-grade APIs for collaborations
- Key milestones ahead are:
  - Publication disclosure in 4Q2020 on the anti-cancer effects and anti-M2M effects of C010DS-Zn
  - *In vivo* tests against live COVID19 strains

-END-