

TRANSLATIONAL ONCOLOGY SUMMIT 2020

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Fast-tracking C010DS-Zn: PARP1-agonist targeting cancer and M2-like macrophages



24 Sept 2020, 10PM (UTC+8)

Jinhyuk Fred Chung, PhD

CSO @ Xylonix

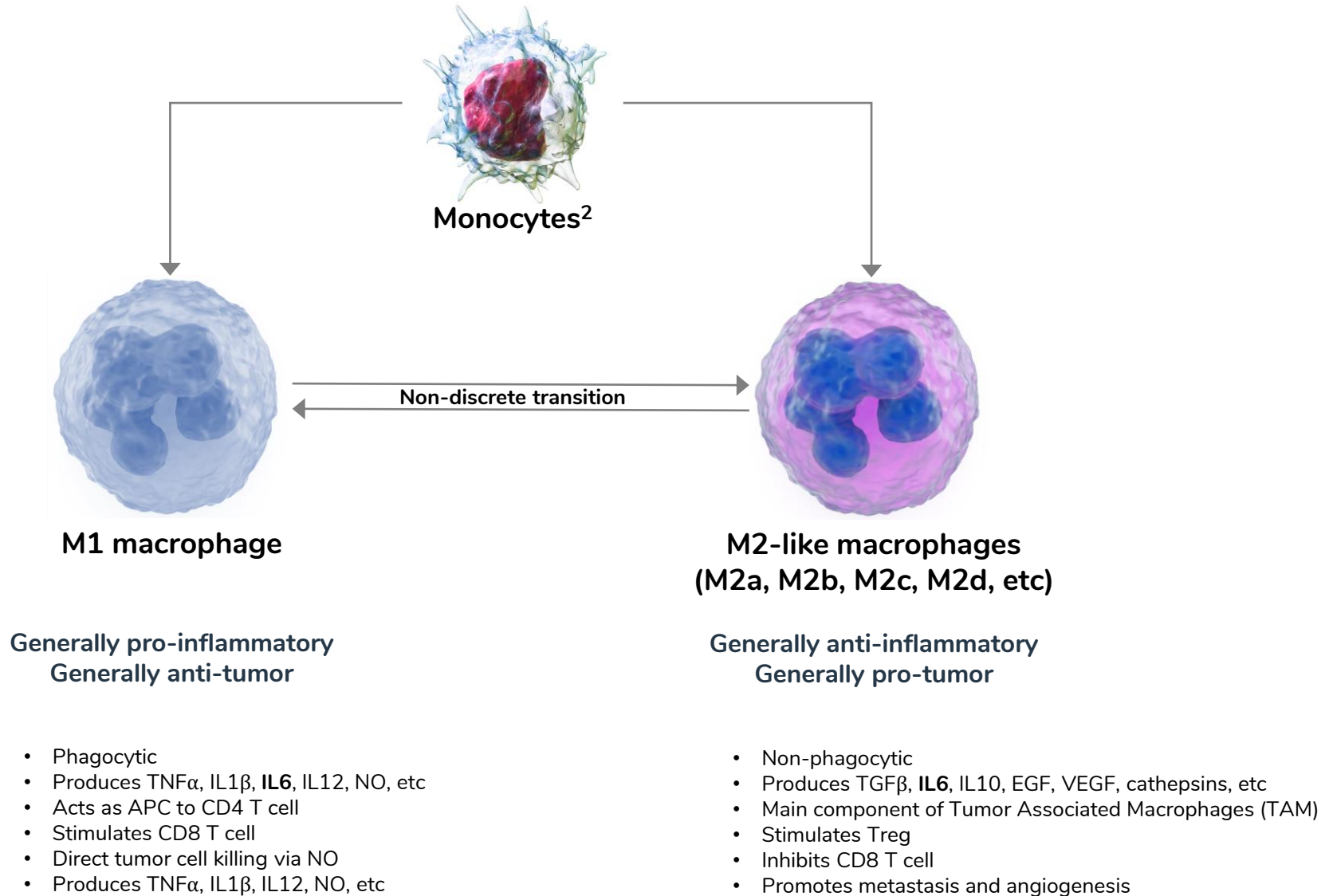
Overview

- 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 hyperactivated 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 macrophages¹



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 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 Lan¹, Li Sun², Feng Xu¹, Lu Liu¹, Fuqing Hu¹, Da Song¹, Zhenlin Hou¹, Wei Wu¹, Xuelai Luo¹, Jing Wang², Xianglin Yuan², Junbo Hu¹, and Guihua Wang¹

Article | [Open Access](#) | Published: 16 May 2019

M2 macrophages promote NSCLC metastasis by upregulating CRYAB

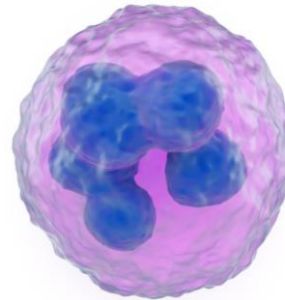
Zhe Guo, Jing Song, Junxia Hao, Hui Zhao, Xiaohui Du, Encheng Li, Yanbin Kuang, Fuquan Yang, Wei Wang , Jiong Deng & Qi Wang

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

J Hematol Oncol. 2017; **10**: 36. PMID: PMC5286803
Published online 2017 Feb 1. doi: [10.1186/s13045-017-0408-0](https://doi.org/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 Chen¹, Siyuan Zhang¹, Qizhi Wang², and Xianbo Zhang^{2#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 Russo¹, Massimo Moro², Michele Sommariva³, Valeria Cancila⁴, Mattia Boeri²,

Trends in Cancer **CellPress REVIEWS**

Opinion

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

Jacob J. Adashek¹, Ishwaria M. Subbiah², Ignacio Matos³, Elena Garralda³, Arjun K. Menta⁴, Dhakshina Moorthy Ganeshan², and Vivek Subbiah ^{2,*}

Severe COVID19 and MSI-C post COVID19

bioRxiv preprint doi: <https://doi.org/10.1101/2020.07.13.190140>; this version posted July 13, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 Manz⁵, Jurjan Aman⁵,

bioRxiv preprint doi: <https://doi.org/10.1101/2020.09.17.300996>; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a [CC-BY-NC-ND 4.0 International license](#).

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

2

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

bioRxiv preprint doi: <https://doi.org/10.1101/2020.07.13.190140>; this version posted July 13, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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Willianne Hoepel^{1,2†}, Hung-Jen Chen^{3†}, Sona Allahverdiyeva^{1,2,4}, Xue Manz⁵, Jurjan Aman⁵,

bioRxiv preprint doi: <https://doi.org/10.1101/2020.09.17.300996>; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

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 6 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** (Hoepel et al., 13 July 2020).
- Hoepel 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

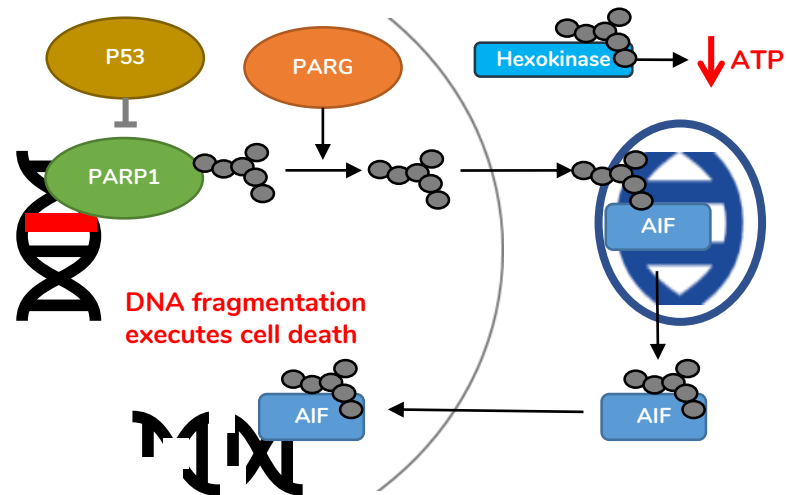


C010DS-Zn: First-in-Class parthanatos inducer against cancer and M2M

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 Zn²⁺ pharmacology toward selective parthanatos induction in the target cells, from C005D-Zn to C010DS-Zn with varying potency
- Zn²⁺ pharmacology was chosen for its simultaneous inhibition of apoptotic caspases¹ 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 Shen¹, Yingwen Hu^{1,2}, Karson S. Putt², Sunil Singhal³, Haiyong Han⁴, Daniel W. Visscher⁵, Linda M. Murphy⁶ 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](https://doi.org/10.1038/srep41340)

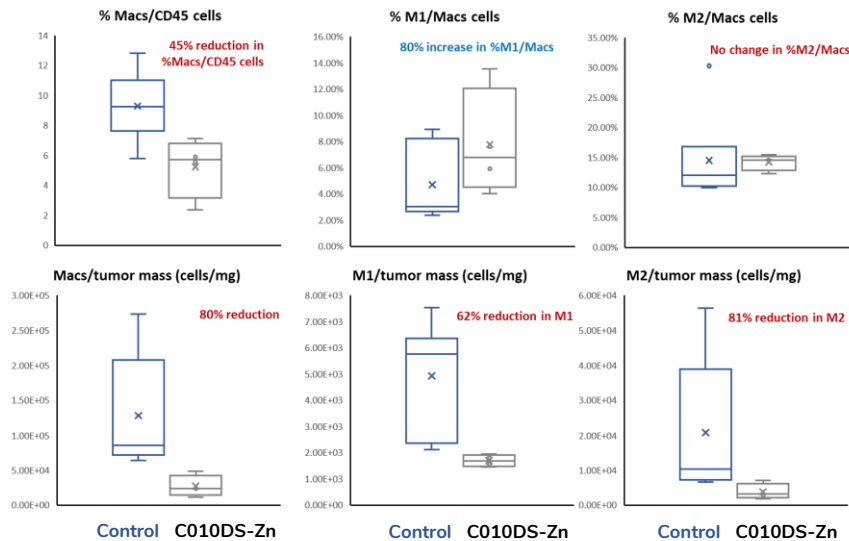
C010DS-Zn takes the center stage post COVID19

Xylonix parthanatos agents at a glance

	C005D-Zn	C008D-Zn	C010DS-Zn
In vitro anti-cancer IC ₅₀ (24h) ¹	290 μM Zn ²⁺	90 μM Zn ²⁺	16 μM Zn ²⁺
In vitro anti-cancer IC ₅₀ (48h) ¹	63 μM Zn ²⁺	44 μM Zn ²⁺	0.7 μM Zn ²⁺
In vitro anti-cancer IC ₉₅ (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)	-/-	-/-	-/-

²Basal serum Zn²⁺ level in healthy individuals is about 150 μM

M2M reduction in tumor by C010DS-Zn³



Xylonix response to the pandemic –fast track C010DS-Zn

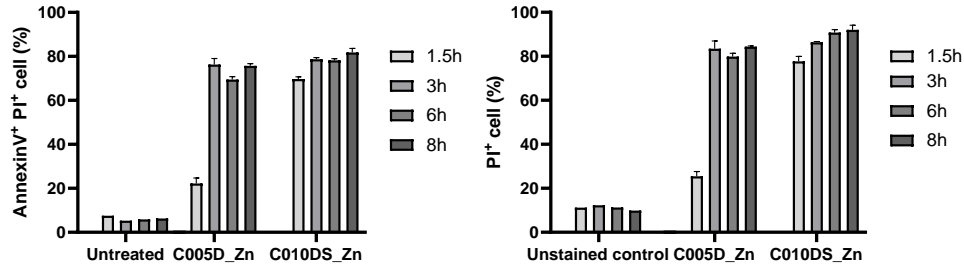
- Prior to the pandemic, C005D-Zn development was prioritized for its excellent safety profiles
- The pandemic brought forth an emergent need for an effective anti-M2M agent, lifting the potential regulatory concerns for C010DS-Zn's capacity to reduce M2M
- Given the clinical and regulatory bottleneck 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 **Invitroque** 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

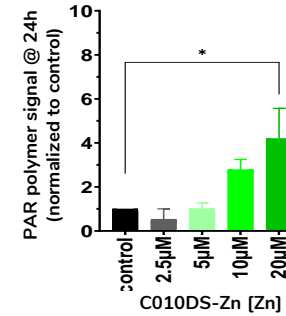
Breakthrough advantages of Xylonix parthanatos agents against cancer

Rapid target parthanatos in hours¹

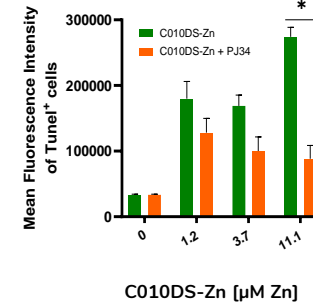
In vitro kinetics of a cancer cell line parthanatos by C005D-Zn or C010DS-Zn @ 4X IC50



PARP agonism by C010DS-Zn

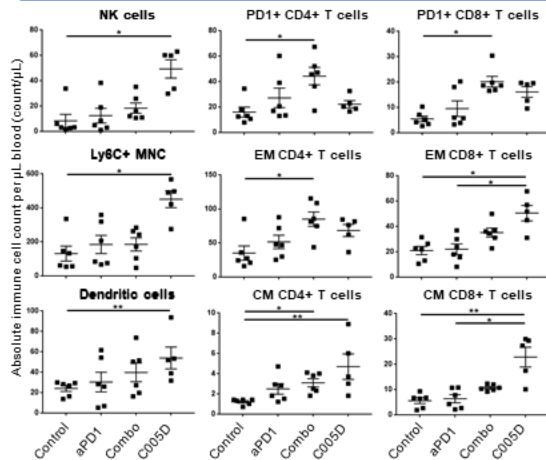


TUNEL signal intensity



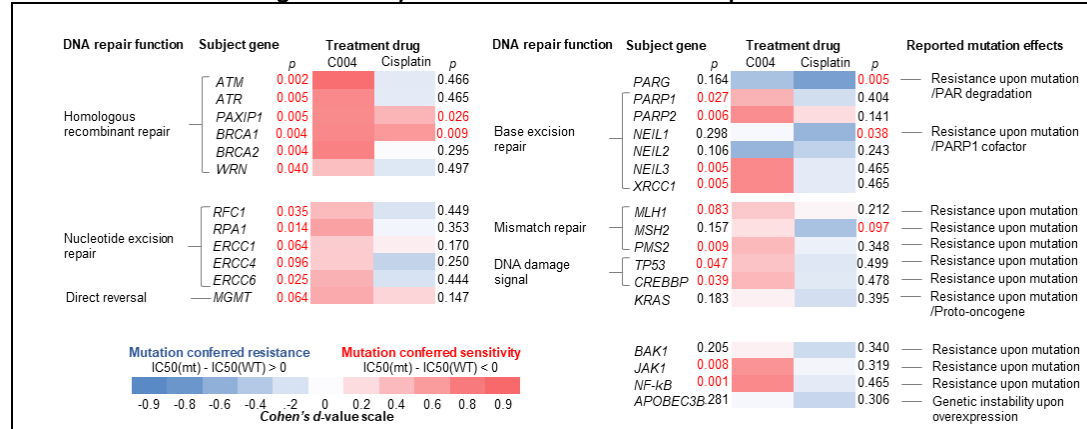
Immune response initiation potential²

Peripheral blood leukocytes in CT26 bearing Balb-c mice



Broad precision indication potential³

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

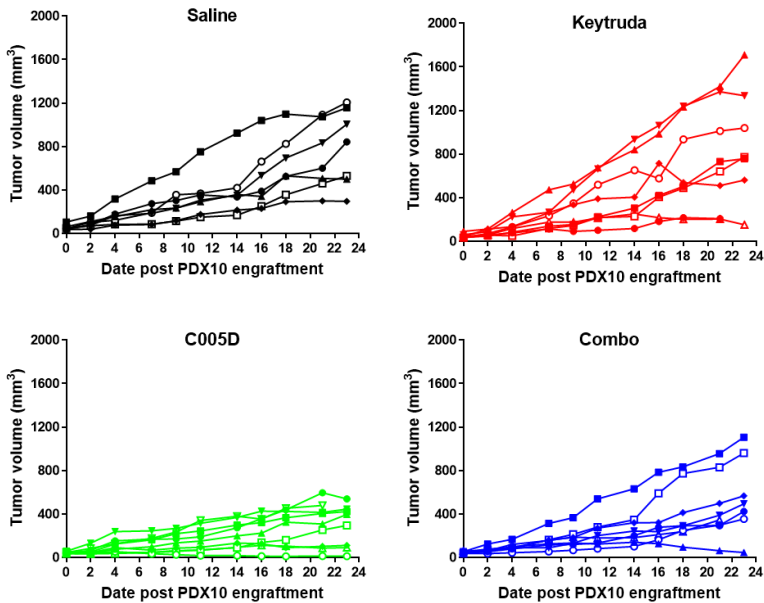
“Gaining a clear understanding of how a drug works before it enters clinical trials is the intelligent route to drug discovery and could increase the likelihood for drug success.”

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

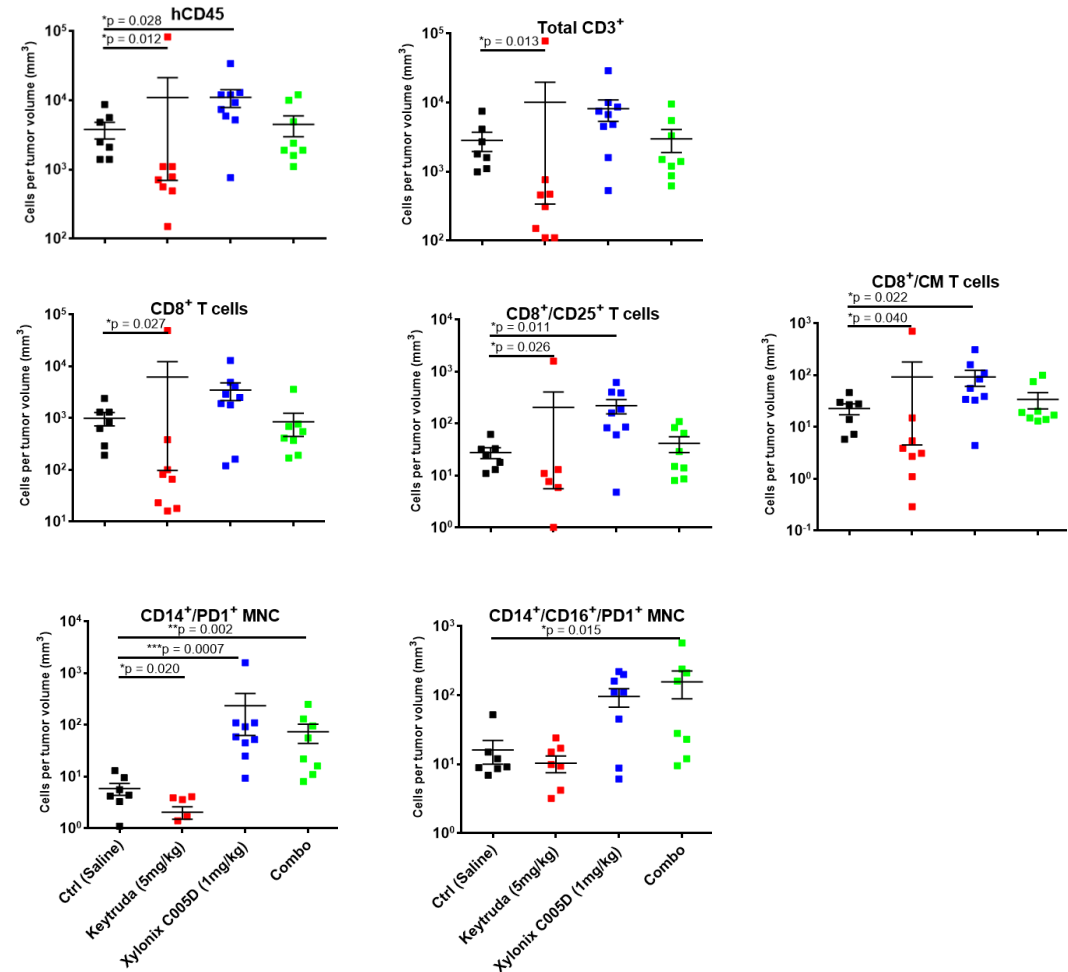
Tumor growth kinetics of HCC on HuMice



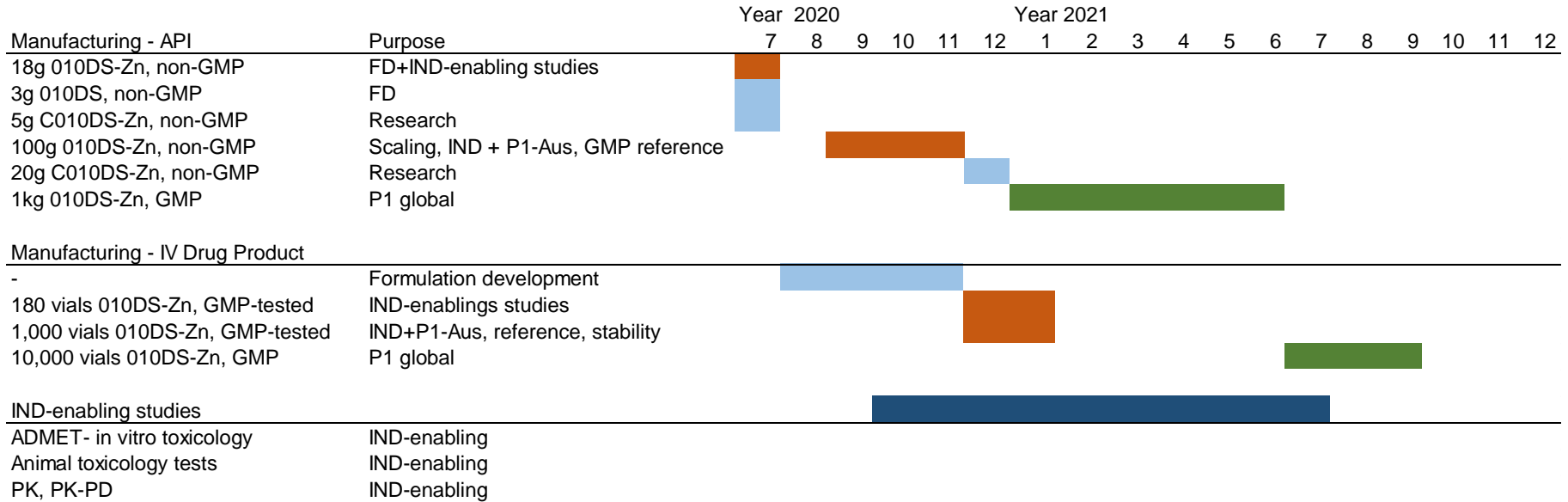
A direct combination study using humanized mice between C005D-Zn and pembrolizumab revealed deleterious effects of macrophage immune response initiation when using pembrolizumab

- 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 interactions¹
- This became the basis of our focus on combination studies with pexidartinib and C010DS-Zn development

Characterization of tumor infiltrating immunity



Fast track development progress on C010DS-Zn



- 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-