APREA THERAPEUTICS

Precision Oncology Through Synthetic Lethality

Aprea Therapeutics KOL Event

June 24, 2024

Forward-Looking Statements

Certain information contained in this presentation includes “forward-looking statements”, within the meaning of Section 27A of the Securities Act of 1933, as amended, and Section 21E of the Securities Exchange Act of 1934, as amended, related to our clinical trials, regulatory submissions and strategic plans. We may, in some cases use terms such as “predicts,” “believes,” “potential,” “continue,” “anticipates,” “estimates,” “expects,” “plans,” “intends,” “may,” “could,” “might,” “likely,” “will,” “should” or other words that convey uncertainty of the future events or outcomes to identify these forward-looking statements. The forward-looking statements are based on current beliefs and expectations of our management team that involve risks, potential changes in circumstances, assumptions, and uncertainties. Any or all of the forward-looking statements may turn out to be wrong or be affected by inaccurate assumptions our management team might make or by known or unknown risks and uncertainties. These forward-looking statements are subject to risks and uncertainties including, without limitation, risks related to the success and timing of our clinical trials or other studies and the other risks set forth in our filings with the U.S. Securities and Exchange Commission, including our Annual Reports on Form 10-K and Quarterly Reports on Form 10-Q. Forward-looking statements regarding our product candidates are also subject to additional risks and uncertainties, including without limitation, with respect to: our dependence on additional financing to fund our operations and complete the development and commercialization of our product candidates, and the risks that raising such additional capital may restrict our operations or require us to relinquish rights to our technologies or product candidates; our limited history and preclinical status of the assets we acquired from Atrin Pharmaceuticals Inc.; our business plan or the likelihood of the successful implementation of such business plan; the timing of initiation of planned clinical trials for our product candidates; the future success of such trials; the successful implementation of our research and development programs and collaborations and the interpretation of the results and findings of such programs and collaborations and whether such results are sufficient to support the future success of our product candidates; the success, timing and cost of our anticipated clinical trials for our current product candidates; the timing of initiation, futility analyses, data presentation, reporting and publication and receipt of interim results (including, without limitation, any preclinical results or data); any statements about our understanding of product candidates mechanisms of action and interpretation of preclinical and early clinical results from its clinical development programs and any collaboration studies; and other factors, including legislative, regulatory, political and economic developments not within our control. For all these reasons, actual results and developments could be materially different from those expressed in or implied by our forward-looking statements. You are cautioned not to place undue reliance on these forward-looking statements, which are made only as of the date of this presentation. We undertake no obligation to update such forward-looking statements to reflect subsequent events or circumstances, except to the extent required by law or regulation.

Today's Speakers

Key Opinion Leaders

Joseph Vacca, PhD

Eric Brown, PhD

Aprea Management Team

Oren Gilad, PhD
President and CEO

Nadeem Mirza, MD, MPH
Chief Medical Officer

ATRIN

Salarius PHARMACEUTICALS

TRIGR THERAPEUTICS

Perelman School of Medicine University of Pennsylvania

Verastem Oncology

sanofi

abbvie

APREA THERAPEUTICS

Today's Agenda

9:00 – 9:05	**Welcome and Introductions** Dr. Oren Gilad
9:05 – 9:15	**Clinical Overview: Neutropenia and Sepsis of Other WEE1 Inhibitors** Dr. Nadeem Mirza
9:15 – 9:25	**Aprea's Medicinal Chemistry Design Approach to APR-1051 Development** Dr. Joe Vacca
9:25 – 9:35	**APR-1051: Chemistry Design Leads to Potential Best in Class WEE1 Inhibitor** Dr. Eric Brown
9:35 – 9:45	**Summary** Dr. Oren Gilad
9:45 – end	**Q&A**

APREA THERAPEUTICS

Who We Are

We are a biopharmaceutical company focused on developing and commercializing novel synthetic lethality-based cancer therapeutics targeting DDR pathways

Our programs are designed to address significant unmet medical needs

Outstanding team of world-class scientific leaders

APREA THERAPEUTICS

Aprea Therapeutics (NASDAQ: APRE)

Precision Oncology via Novel Synthetic Lethality Therapeutics

All programs address significant unmet medical need, are synergistic with other anticancer therapies, and potentially differentiated in safety and tolerability

ATR Inhibitor: ATRN-119

- First macrocyclic ATR inhibitor
- Highly selective with continuous daily dosing
- Phase 1/2a – Ongoing Dose Escalation
  - Readout 1Q 2025
  - Solid tumor with DDR mutation
- Pre-clinical proof-of-principle
  - Anti-tumor activity at nanomolar concentration
  - Preserved hematologic safety profile

WEE1 Inhibitor: APR-1051

- Best in class, next generation
- Pre-clinical proof-of-principle
  - Highly potent and selective anti-tumor activity
  - Minimal off target effect
  - Ovarian cancer with Cyclin E over expression (OVCAR-3)
  - Stable hematologic function
  - Favorable pharmacokinetics
- First patient dosed June 2024
- Study update 4Q 2024

DDR Inhibitor: Undisclosed

- Lead optimization
- Target identified from our RepliBiom discovery platform

APREA THERAPEUTICS

ATR - Ataxia telangiectasia and Rad3-related
DDR – DNA Damage Response

Today's Take Home Messages

Potential best in class WEE1 inhibitor

01 Same target different drug	Structurally different molecule
02 Similar efficacy observed in vitro	High potency for WEE1 inhibition in vitro
03 Potential higher safety	• Limited off-target inhibition of the PLK family of kinases • PLK1 suppression is associated with increased risk of sepsis
04 Therefore, we anticipate higher therapeutic index	• IND cleared • FDA did not raise sepsis concerns

APREA THERAPEUTICS

Published Data Showing Other WEE1 Inhibitors Are Potent with Limited Selectivity Potentially Implies Off-Target Toxicity

	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 µM (%)		
	WEE1	**PLK1**	**PLK2**	**PLK3**
Zentalis: Azenosertib (ZN-c3)[1]	3.2	79	96	92
AstraZeneca: Adavosertib (AZD-1775)[1,2]	3.9	70	101	91

Note: Head-to-head studies have not been conducted

1. Huang et al, (2021) *J Med Chem*
2. AstraZeneca announced in July 2022 the discontinuation of development of AZD-1775

Clinical Overview:

Neutropenia and Sepsis of Other WEE1 Inhibitors

Dr. Nadeem Mirza

APREA THERAPEUTICS

Azenosertib (ZN-c3) – Partial Clinical Hold on Three Clinical Studies

- On June 18, 2024, the FDA placed 3 clinical studies of azenosertib on a partial clinical hold:
  - ZN-c3-001, a Phase 1 dose-escalation monotherapy study in solid tumors
  - ZN-c3-004 (TETON), a Phase 2 monotherapy study in USC[1]
  - ZN-c3-005 (DENALI), a Phase 2 monotherapy study in PROC[2]
- The partial hold follows two drug-related deaths in 1H 2024 due to presumed sepsis in the DENALI study
- Zentalis also provided details on three additional Grade 5 treatment-related neutropenia/sepsis events in their sponsored solid tumor studies[3]

1. Uterine serous carcinoma
2. Platinum-resistant ovarian cancer
3. Zentalis, Azenosertib Clinical Development Update, June 18, 2024

APREA THERAPEUTICS

Sepsis Has Also Been Reported With Astra Zeneca's Adavosertib

Treatment	Phase (n)	Tumor Type	AEs of Interest With Respect to Sepsis	Sepsis Cases
Adavosertib Monotherapy	Phase 1 (25)[1]	Solid tumors	Neutropenia 40%, Grade ≥3 16% FN[2] 4%	1 fatal sepsis
Adavosertib Monotherapy	Phase 2 (80)[3]	Recurrent high grade serous ovarian cancer with cyclin E1 overexpression with and without gene amplification	Dose reduction was required in 36 (45%) patients, mostly for neutropenia (or diarrhea)	3 fatal sepsis
Adavosertib Monotherapy	Phase 2b (109)[4]	USC	Neutropenia Grade≥3 21%	7 sepsis cases: • 5 recovered • 2 fatal 5 sepsis events associated with Grade 4 neutropenia (includes 2 fatal)

Note: Head-to-head studies have not been conducted

1. Phase I Study of Single-Agent AZD1775, a Wee1 Kinase Inhibitor, in Patients With Refractory Solid Tumors _ Do et al _ Journal Of Clinical Oncology_ Volume 33 _ Number 30 _ October 20 2015
2. Febrile neutropenia
3. IGNITE: A phase II signal-seeking trial of Adavosertib targeting recurrent high-grade, serous ovarian cancer with cyclin E1 overexpression with and without gene amplification. Au-Yeung et al, Int J Gynecol Cancer 2023;33(Suppl 4):A1–A278
4. A Phase 2b, Open-label, Single-arm, Multi-centre Study Assessing the Efficacy and Safety of Adavosertib as Treatment for Recurrent or Persistent Uterine Serous Carcinoma (ADAGIO)_Astra Zeneca_ Clinical Study Report Synopsis_ 12 December 2022

APREA THERAPEUTICS

PLK1 Inhibitors Are Associated with Severe Neutropenia/Sepsis in Clinical Studies

Volasertib (BI6727), a PLK1 Inhibitor, Boehringer Ingelheim

Phase 1 trial[1]: Most common drug-related AE in schedule A[2] was neutropenia (56.3%). Gr≥3 neutropenia in 13 of 20 patients (65%) treated with doses (300 and 350mg) similar to a later Phase 3 study.

Phase 3 trial[3]: AML patients ineligible for intensive chemotherapy, randomized
Arm 1: Volasertib[4] with low-dose cytarabine (LDAC[5]); n = 444
Arm 2: LDAC[5] with placebo; n = 222

	Placebo + LDAC[3] (n=222) Grade 3 - 5	Volasertib + LDAC[3] (n=439) Grade 3 - 5
Neutropenia	36 (16.2%)	128 (29.3%)
Febrile neutropenia[4]	63 (28.4%)	258 (58.8%)
Sepsis	8 (3.6%)	49 (11.2%)

All AEs leading to death were reported with a higher frequency in the Volasertib + LDAC arm (31.2%) than in the Placebo + LDAC arm (18.0%), potentially driven by a higher incidence of infections and infestations (17.1% versus 6.3%)

MK-1496, a PLK1 Inhibitor, Merck

Phase 1 trial[7]: One of the most frequent Gr ≥3 AEs was neutropenia (35%)
"Neutropenia caused by MK-1496 is a mechanism-based effect of PLK1 inhibition"

1. A phase I study of two dosing schedules of volasertib (BI 6727), an intravenous polo-like kinase inhibitor, in patients with advanced solid malignancies_Lin et al_ *British Journal of Cancer* (2014) 110, 2434–2440
2. A 2-h infusion on day 1 in a 3-week schedule
3. Adjunctive Volasertib in Patients With Acute Myeloid Leukemia not Eligible for Standard Induction Therapy –A Randomized, Phase 3 Trial_ Dohner et al_European Hematology Association_ *Hemasphere.*
   2021 Aug; 5(8), including its Supplemental Digital Content
4. 350mg IV on days 1 and 15 in 4-wk cycles
5. Low-dose cytarabine: 20mg SC, twice daily, days 1–10
6. Neutropenia complicated by infections
7. A first-in-human phase I dose-escalation study of MK-1496, first-in-class orally available novel PLK1 inhibitor, in patients with advanced solid tumors_Murakami et al_ASCO 2011

APREA THERAPEUTICS

APR -1051

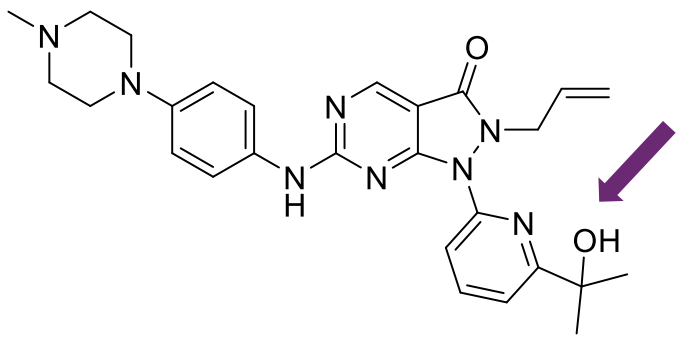
Aprea's Medicinal Chemistry Design Approach to APR-1051 Development

Dr. Joe Vacca

APREA THERAPEUTICS

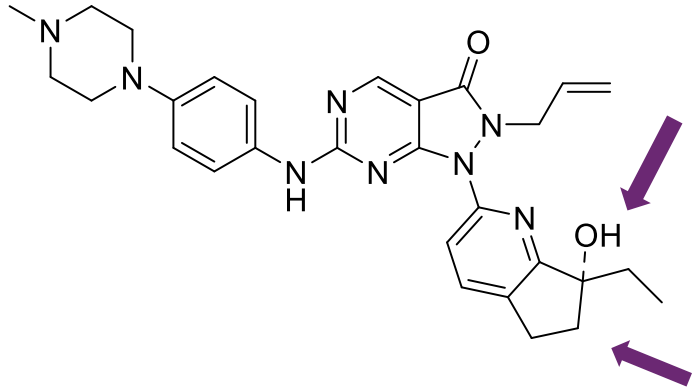
AZD1775 and ZN-c3 Inhibit WEE1 and PLK1 Similarity

Asn 431 active site In WEE1 is similar Asp 194 In PLK1 site



AZD1775[1]

WEE1 IC50 = 3.9 nM
PLK1 IC50 = 3.0 nM



Binds to Asn 431
In WEE 1 site
Similar Asp in PLK1

Small difference

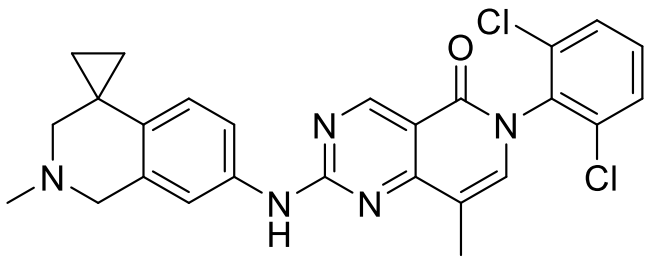
ZN-c3[2]

WEE1 IC50 = 3.2 nM
PLK1 = IC50 92.1 nM

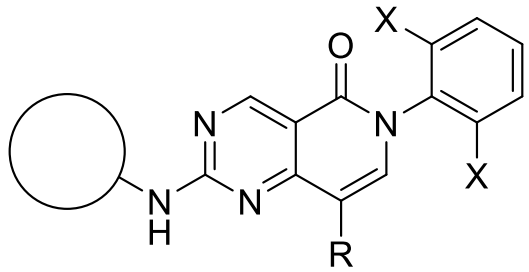
- AZD1775 had equal activity against PLK1 and WEE1, likely through alcohol binding to Asp (Asp 194) in PLK site
- ZN-c3 structure very similar to AZD1775 and binds to PLK1, 2 and 3

1. J. Med. Chem. 2021, 64, 13004-13024
2. Data on File

Aprea Design – Eliminate Alcohol to Increase Selectivity and Maintain Efficacy



Compound 36*
WEE1 IC50 = 0.5 nM



APR-1051 general scaffold
WEE1 IC50 = 1.8 nM
PLK1 IC50 = 15,900 nM

- Aprea decided to use a different design based on potent literature scaffolds
- Eliminated the alcohol that bound to PLK1 Aspartic acid group
- As expected, APR-1051 had very weak binding to PLK1
- Aprea's APR-1051 maintains WEE1 inhibition at low nano molar IC50

*Bioorganic & Medicinal Chemistry Letters, 29 (2019) 1481-1486

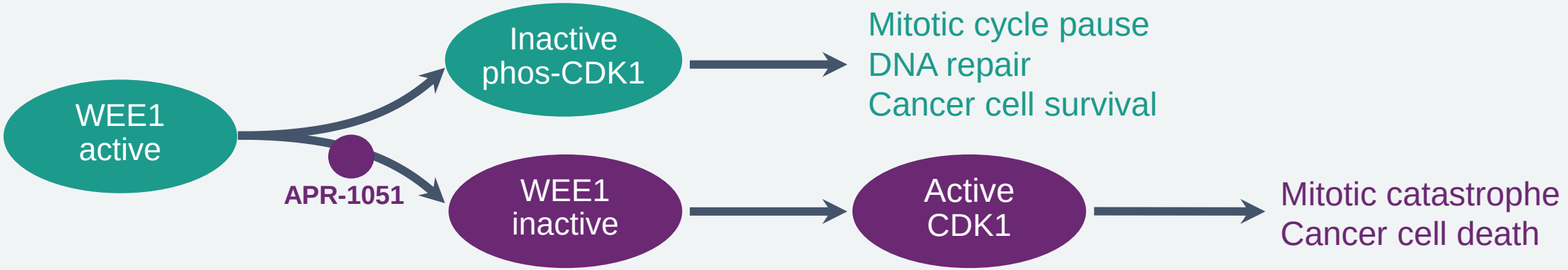
APR -1051

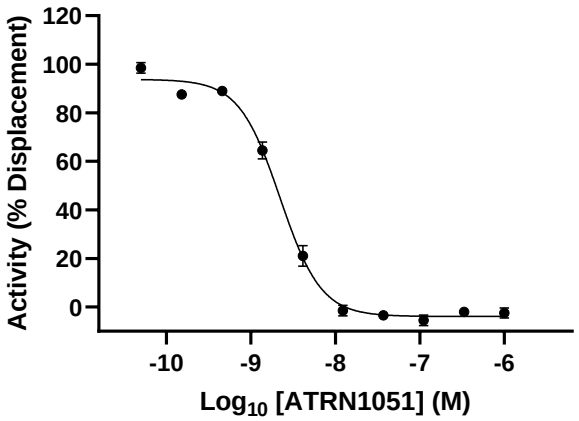
Chemistry Design Leads to Potential Best in Class WEE1 Inhibitor

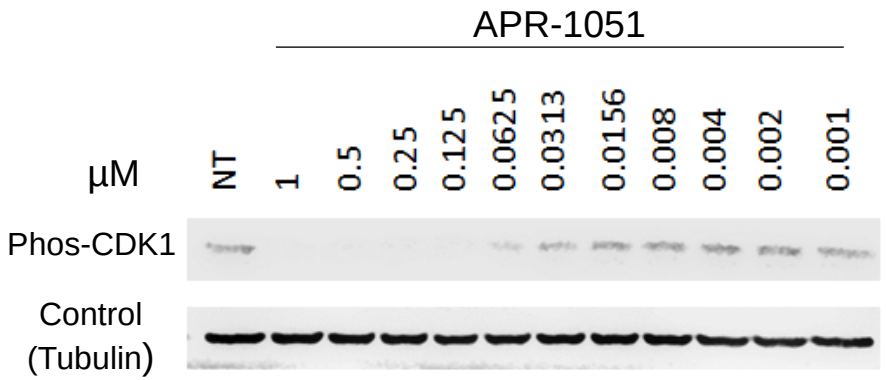
Dr. Eric Brown

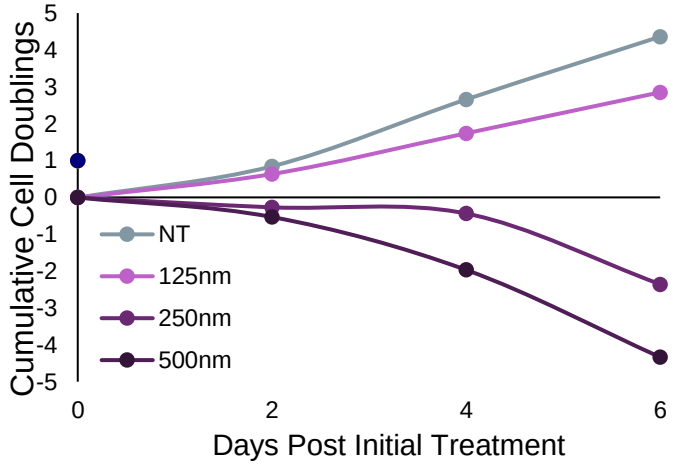
APREA THERAPEUTICS

WEE1 Inhibitor – APR-1051
Mechanism of Action – Prevent CDK1 Phosphorylation by WEE1 Kinase









APR-1051 binds to WEE1

…inhibits its biological activity…

…and triggers mitotic catastrophe and cancer cell death

Phos-CDK1- Phosphorylated Cyclin Dependent Kinase 1
Data on file

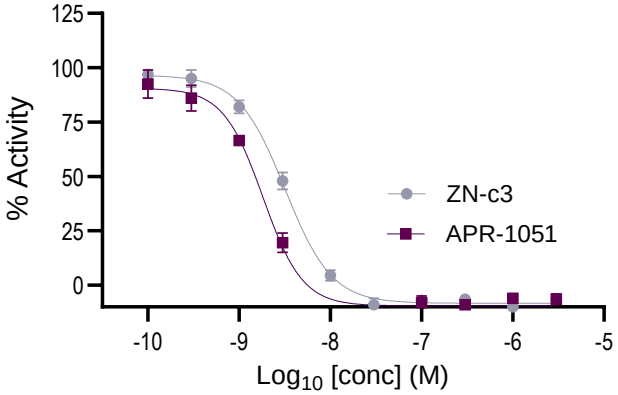
APR-1051: Potentially Best-in-Class WEE1 Inhibitor

APR-1051 is a potent WEE1i that does not substantially inhibit PLK1, PLK2 or PLK3

On-target WEEi activity

Off-target inhibition of PLK1, PLK2 and PLK3

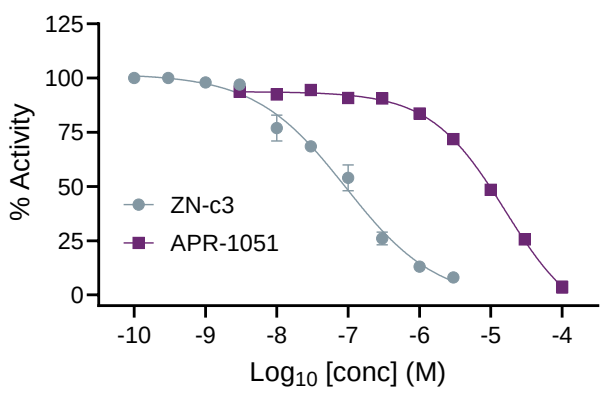
WEE1 IC50 Determination



ZN-c3 = 3.2 nM
APR-1051 = 1.8 nM

WEE1 Inhibition
IC50 similar to ZN-c3

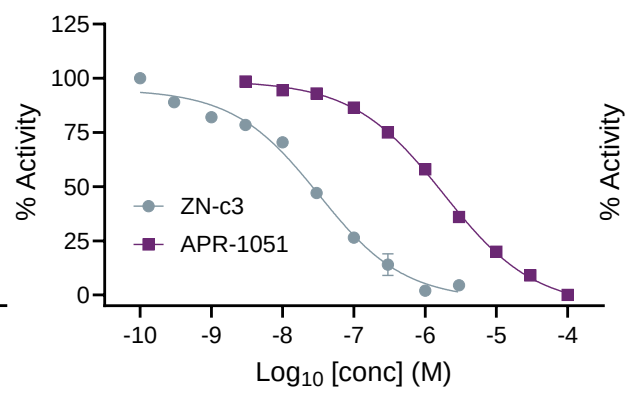
PLK1 IC_{50} Determination



ZN-c3 = 92.1 nM
APR-1051 = 15,900 nM

PLK1 Inhibition
IC50, >150-fold difference

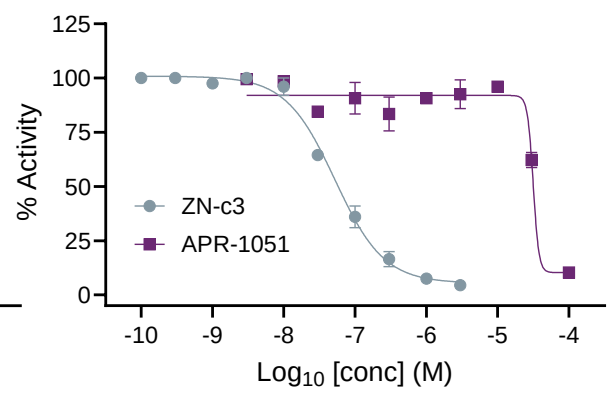
PLK2 IC_{50} Determination



ZN-c3 = 32.0 nM
APR-1051 = 1,800 nM

PLK2 Inhibition
IC50, >50-fold difference

PLK3 IC_{50} Determination

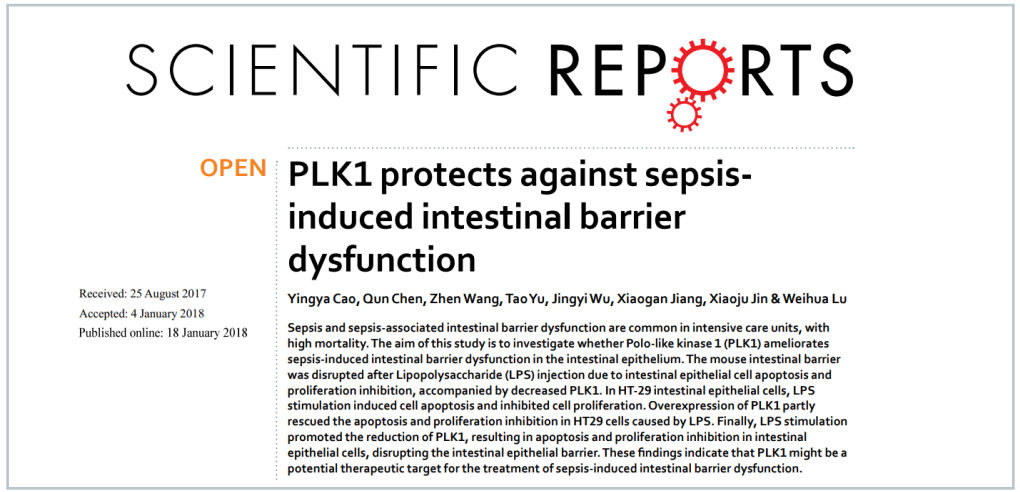


ZN-c3 = 52.2 nM
APR-1051 = 31,600 nM

PLK3 Inhibition
IC50, >600-fold difference

Data is consistent with that reported in Huang et al, (2021) *J Med Chem.* AstraZeneca announced the discontinuation of the development of AZD-1775 in July 2022.

Studies Show PLK1 Suppression is Associated with Sepsis-Induced Loss of Intestinal Barrier Function



SCIENTIFIC REPORTS

OPEN

PLK1 protects against sepsis-induced intestinal barrier dysfunction

Received: 25 August 2017
Accepted: 4 January 2018
Published online: 18 January 2018

Yingya Cao, Qun Chen, Zhen Wang, Tao Yu, Jingyi Wu, Xiaogan Jiang, Xiaoju Jin & Weihua Lu

Sepsis and sepsis-associated intestinal barrier dysfunction are common in intensive care units, with high mortality. The aim of this study is to investigate whether Polo-like kinase 1 (PLK1) ameliorates sepsis-induced intestinal barrier dysfunction in the intestinal epithelium. The mouse intestinal barrier was disrupted after Lipopolysaccharide (LPS) injection due to intestinal epithelial cell apoptosis and proliferation inhibition, accompanied by decreased PLK1. In HT-29 intestinal epithelial cells, LPS stimulation induced cell apoptosis and inhibited cell proliferation. Overexpression of PLK1 partly rescued the apoptosis and proliferation inhibition in HT29 cells caused by LPS. Finally, LPS stimulation promoted the reduction of PLK1, resulting in apoptosis and proliferation inhibition in intestinal epithelial cells, disrupting the intestinal epithelial barrier. These findings indicate that PLK1 might be a potential therapeutic target for the treatment of sepsis-induced intestinal barrier dysfunction.

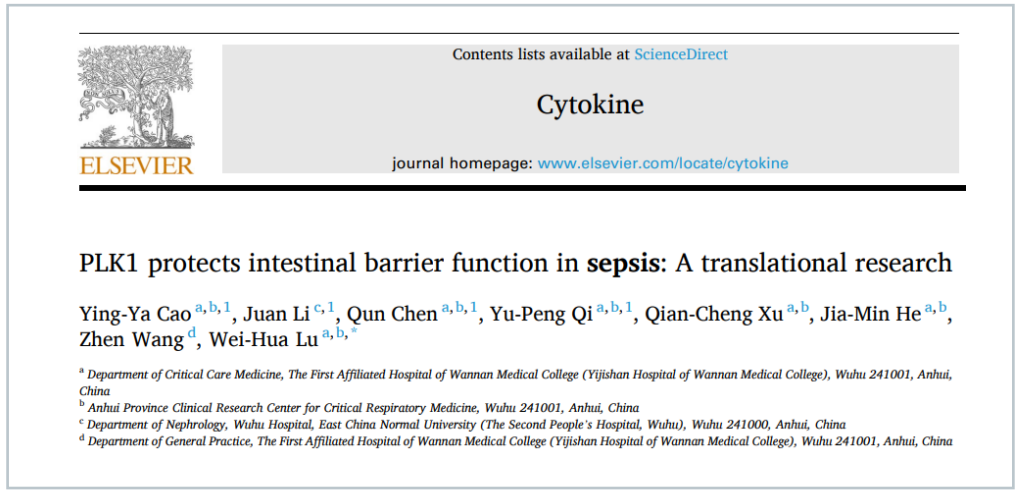
Cao et al. Molecular Medicine (2022) 28:163
https://doi.org/10.1186/s10020-022-00597-z

Molecular Medicine

RESEARCH ARTICLE Open Access

PLK1 protects intestinal barrier function during sepsis by targeting mitochondrial dynamics through TANK-NF-κB signalling

Ying-Ya Cao[1,2†], Yuan Zhang[1†], Wuyun Gerile[1†], Yan Guo[1], Li-Na Wu[1], Li-Li Wu[1], Kai Song[1], Wei-Hua Lu[2] and Jian-Bo Yu[1*]



Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

PLK1 protects intestinal barrier function in **sepsis**: A translational research

Ying-Ya Cao[a,b,1], Juan Li[c,1], Qun Chen[a,b,1], Yu-Peng Qi[a,b,1], Qian-Cheng Xu[a,b], Jia-Min He[a,b], Zhen Wang[d], Wei-Hua Lu[a,b,*]

[a] Department of Critical Care Medicine, The First Affiliated Hospital of Wannan Medical College (Yijishan Hospital of Wannan Medical College), Wuhu 241001, Anhui, China
[b] Anhui Province Clinical Research Center for Critical Respiratory Medicine, Wuhu 241001, Anhui, China
[c] Department of Nephrology, Wuhu Hospital, East China Normal University (The Second People's Hospital, Wuhu), Wuhu 241000, Anhui, China
[d] Department of General Practice, The First Affiliated Hospital of Wannan Medical College (Yijishan Hospital of Wannan Medical College), Wuhu 241001, Anhui, China

Received: 15 February 2021 | Revised: 25 April 2021 | Accepted: 16 May 2021
DOI: 10.1002/cbin.11633

RESEARCH ARTICLE

Cell Biology International WILEY

LncRNA DANCR improves the dysfunction of the intestinal barrier and alleviates epithelial injury by targeting the miR-1306-5p/PLK1 axis in sepsis

Zhen Wang[1] | Changshun Zhong[1] | Yingya Cao[1] | Hongzhen Yin[1] | Guanggui Shen[1] | Weihua Lu[1] | Wei Ding[2]

1. PLK1 protects against sepsis-induced intestinal barrier dysfunction, Cao et al, *Scientific Reports* (2018).
2. PLK1 protects intestinal barrier function in sepsis: A translational research, Cao et al, *Cytokine* (2023).
3. PLK1 protects intestinal barrier function in sepsis: A translational research, Cao et al, *Molecular Medicine* (2022).
4. LncRNA DANCR improves the dysfunction of the intestinal barrier and alleviates epithelial injury by targeting the miR-1306-5p/PLK1 axis in sepsis, Wang et al., *Cell Biology International* (2021).

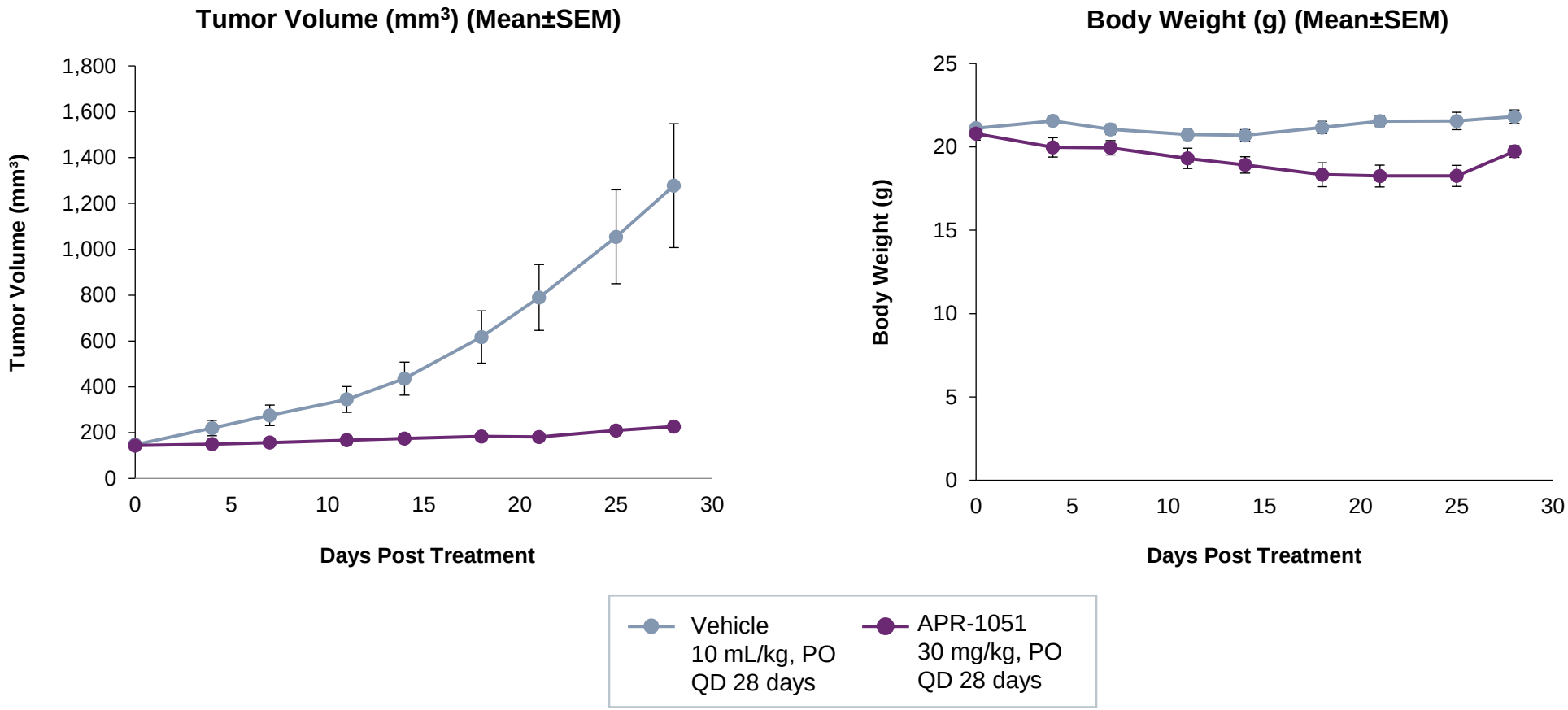
Studies Indicate That PLK1 Protects the Intestinal Barrier

- The intestine plays a crucial role in the pathophysiology of sepsis
  - Intestinal barrier prevents the entry of bacteria and toxins into the circulation
  - Maintenance of the intestinal barrier is critical for limiting the effects of sepsis
- The main component of the intestinal barrier is the epithelial cells of the intestinal mucosa
- Intestinal mucosal barrier stability relies on the balance of proliferation and apoptosis of intestinal epithelial cells
- PLK1 inhibition slowed recovery of intestinal barrier function, causing decreased survival, and overexpression of PLK1 increased barrier function and improved survival
- Multiple recent studies have demonstrated that PLK1 and associated pathways protect intestinal barrier function

1. PLK1 protects against sepsis-induced intestinal barrier dysfunction, Cao et al, *Scientific Reports* (2018).
2. PLK1 protects intestinal barrier function in sepsis: A translational research, Cao et al, *Cytokine* (2023).
3. PLK1 protects intestinal barrier function in sepsis: A translational research, Cao et al, *Molecular Medicine* (2022).
4. LncRNA DANCR improves the dysfunction of the intestinal barrier and alleviates epithelial injury by targeting the miR-1306-5p/PLK1 axis in sepsis, Wang et al., *Cell Biology International* (2021).

APR-1051 Suppresses Tumor Growth While Causing Little Effect on Body Weight

OVCAR Xenograft Tumor Model in Female Nude Mice



N=7 mice per group, APR-1051, exploratory formulation - 30 mg/kg/day

Pre-clinical studies with APR-1051
Data on file

Aprea Therapeutics (NASDAQ: APRE)

Summary

Dr. Oren Gilad

APREA THERAPEUTICS

Summary

Potential best in class WEE1 inhibitor

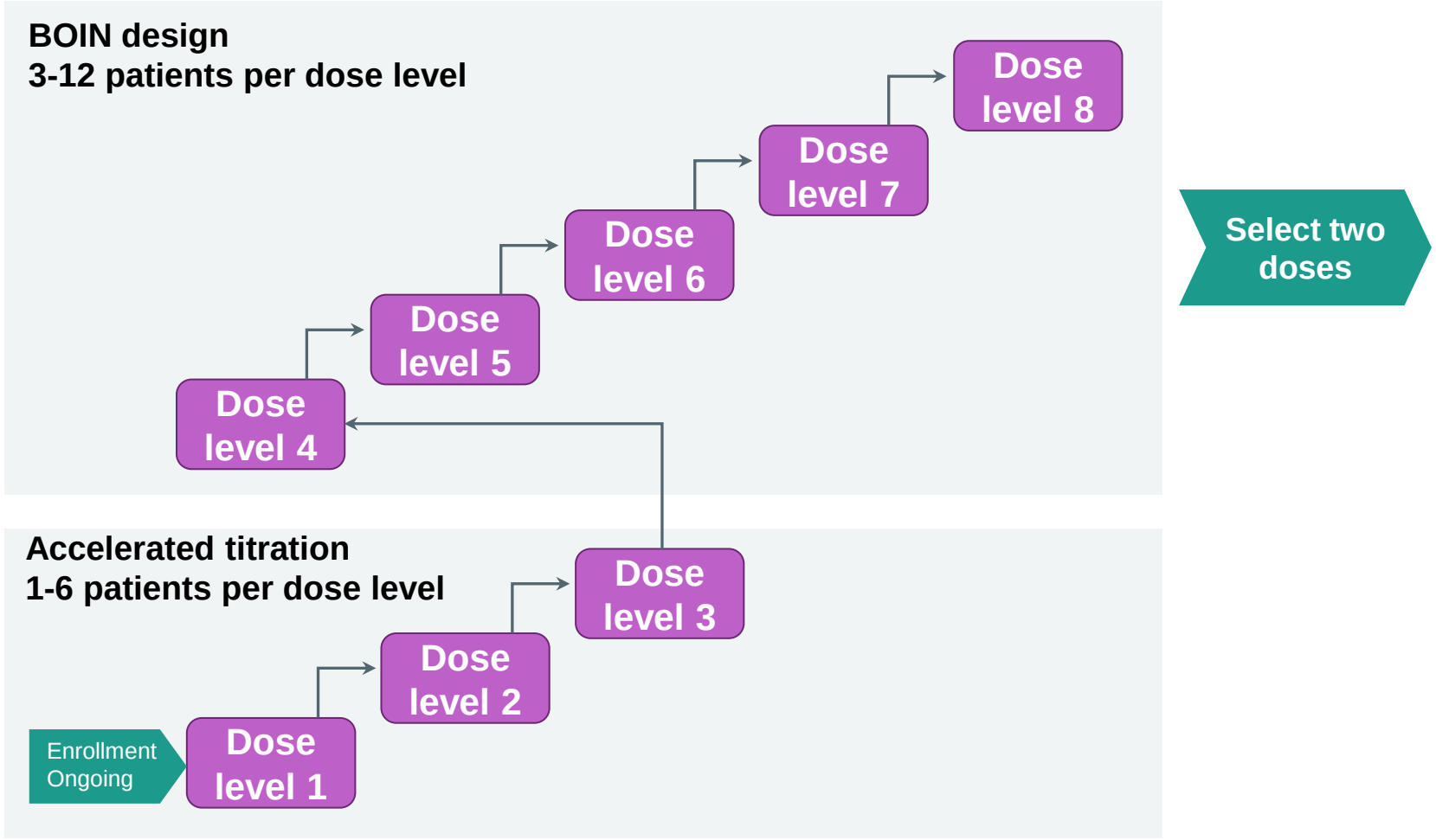
01 Same target different drug	Structurally different molecule
02 Similar efficacy observed in vitro	High potency for WEE1 inhibition in vitro
03 Potential higher safety	• Limited off-target inhibition of the PLK family of kinases • PLK1 suppression is associated with increased risk of sepsis
04 Therefore, we anticipate higher therapeutic index	• IND cleared • FDA did not raise sepsis concerns

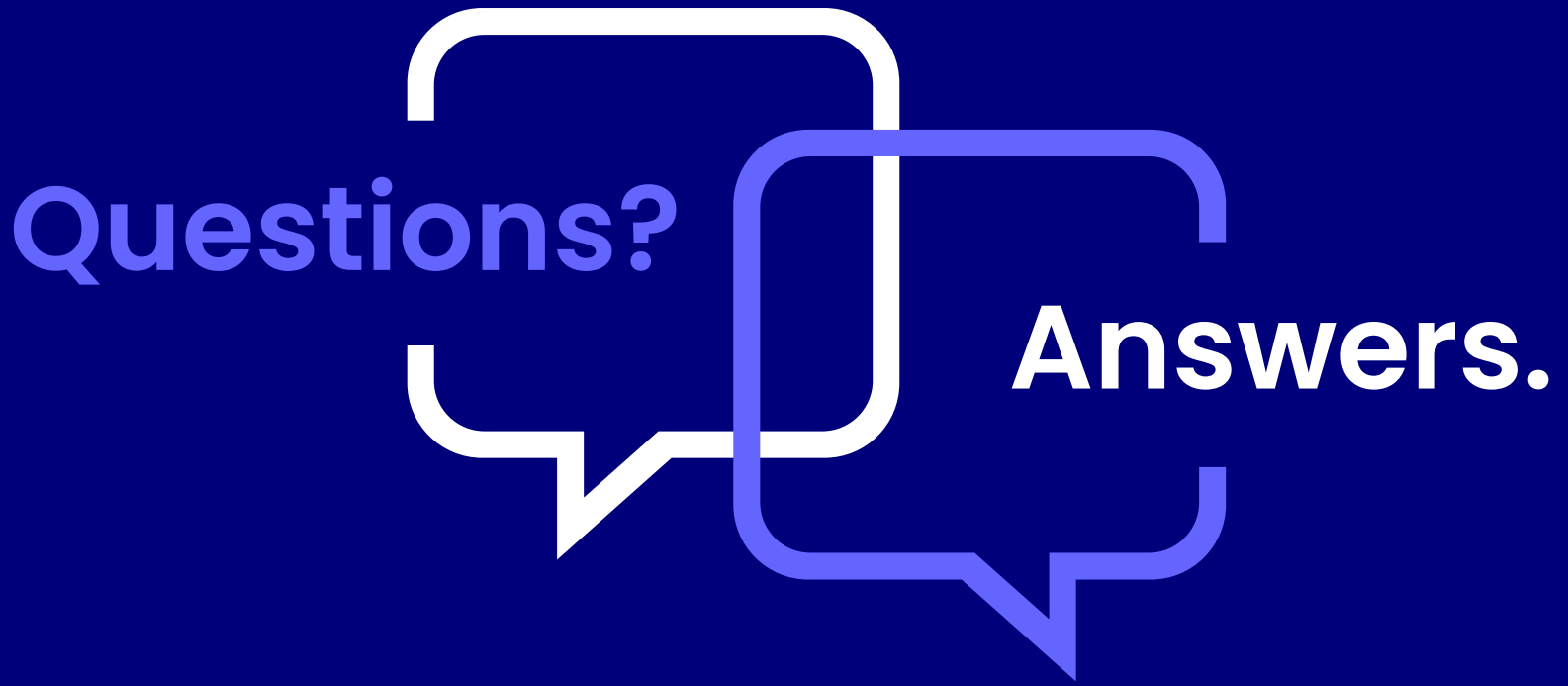
APREA THERAPEUTICS

ACESOT-1051

Part 1 - Single-agent APR-1051 Dose Escalation Study Schema

Up to 39 patients with advanced solid tumors harboring cancer-associated gene alterations:





Q&A Session