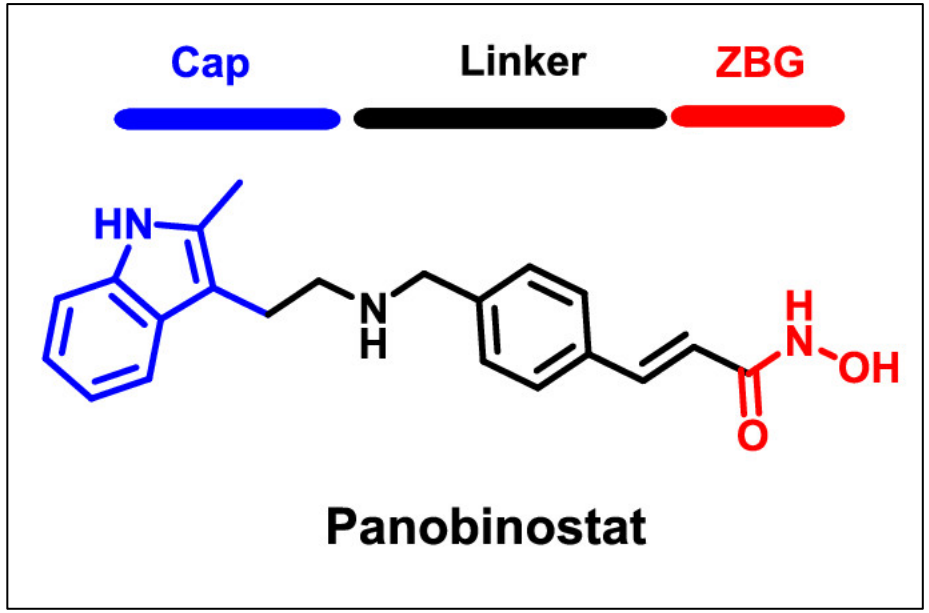
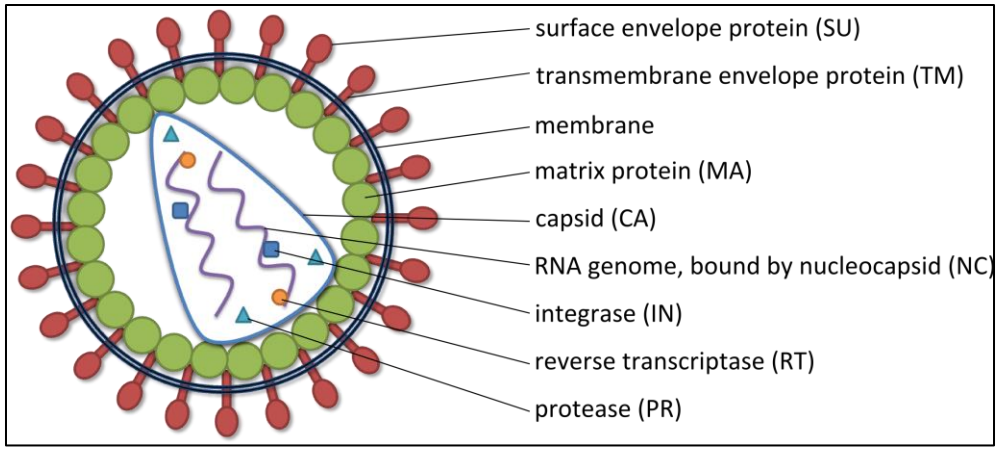
Synthistat

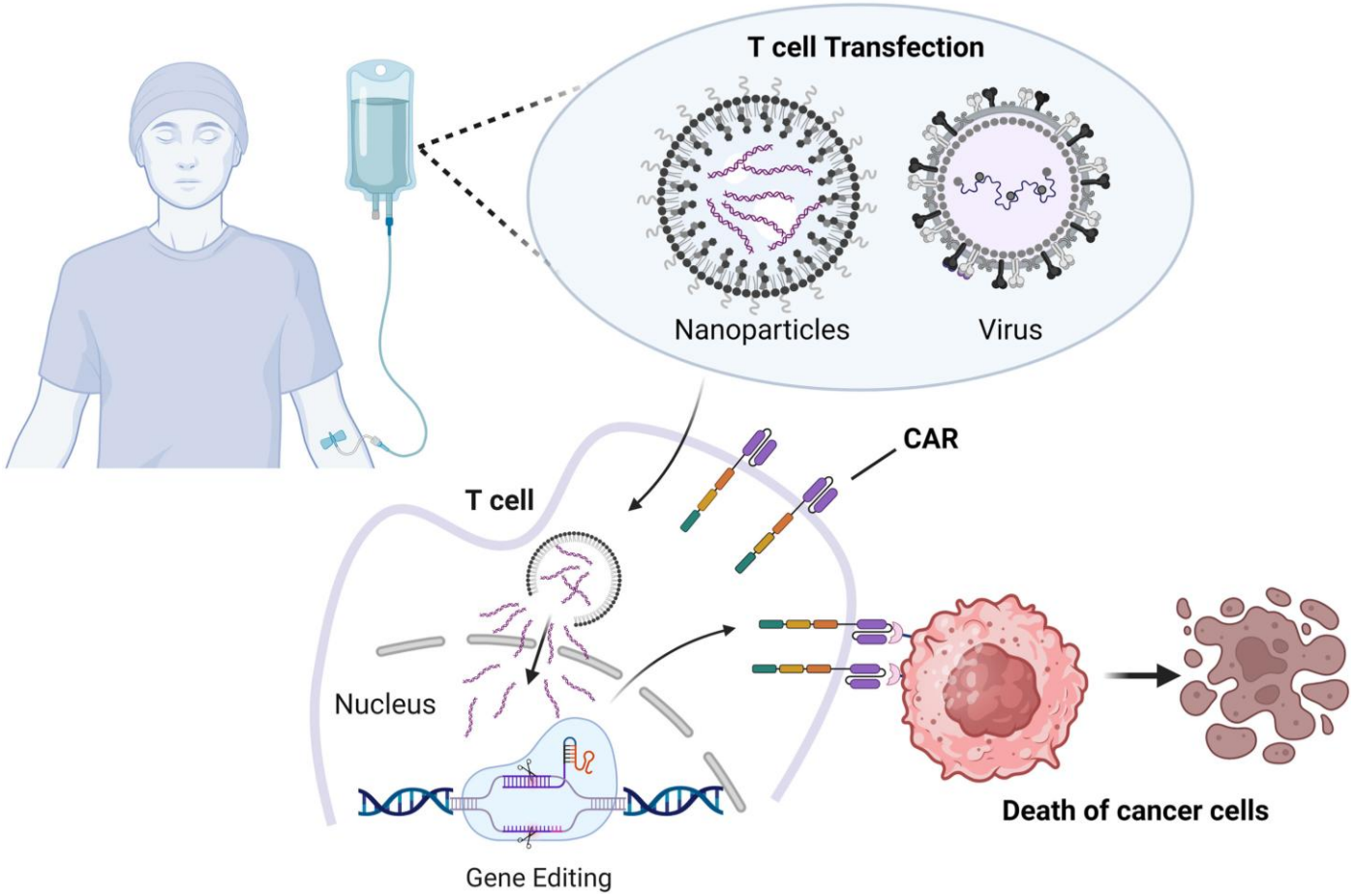
Synthistat

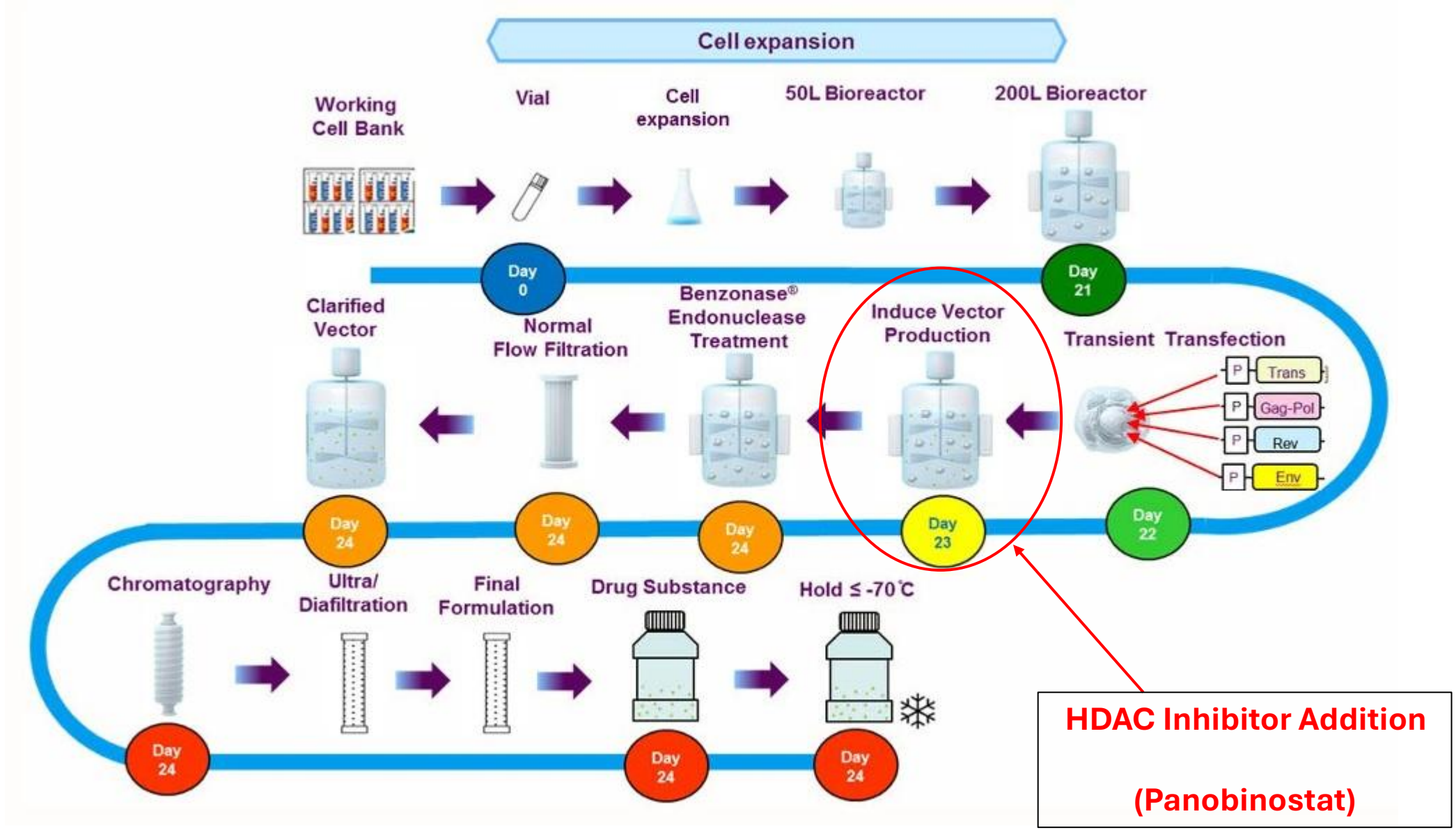
Where pharmaceutical science meets viral vectors





Introduction




Cell expansion
Working Cell Bank
Vial
Cell expansion
50L Bioreactor
200L Bioreactor
Day 0
Day 21
Clarified Vector
Normal Flow Filtration
Benzonase® Endonuclease Treatment
Induce Vector Production
Transient Transfection
P Trans
P Gag-Pol
P Rev
P Env
Day 24
Day 24
Day 24
Day 23
Day 22
Chromatography
Ultra/ Diafiltration
Final Formulation
Drug Substance
Hold ≤ -70˚C
Day 24
Day 24
Day 24
HDAC Inhibitor Addition
(Panobinostat)

Introduction

- HDAC inhibitors, including panobinostat, comprise a class of drug compound with clinical indications for the treatment of oncological malignancies and they are also included within treatment regiments for HIV- 1. The HDAC inhibitor sodium butyrate is also widely used as a production enhancer for clinical lentiviral vectors, as it typically delivers 10-fold improvement in the production of lentiviral vectors

- However, it has recently been discovered by Abdi et al 2025, that the alternative HDAC inhibitor panobinostat performs twice as well as sodium butyrate for lentiviral vector (LVV) production

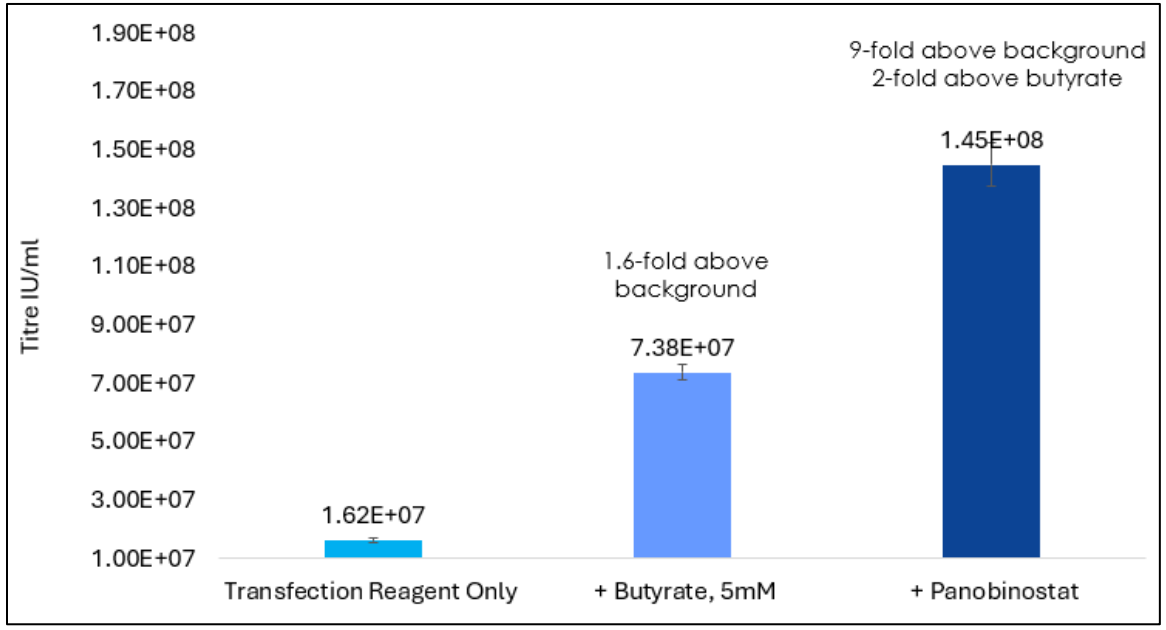


Figure 1, presents mean average (N of 4) of virus titres produced in suspension HEK 293 using panobinostat or sodium butyrate 5 mM, for virus production in suspension HEK 293 cells. Panobinostat increased lentiviral vectors titres on average 8.95-fold greater than titres produced using transfection agent alone and 1.96-fold better than titres produced with sodium butyrate addition. GFP virus was produced using Aldevron lentiviral vector helper plasmids, suspension HEK 293, Sartorius TF Media (1% PS and 3% Glutamax) and Mirus TransIT transfection reagent.

Panobinostat Analogues

Consequently, through studying the structural features of panobinostat which are responsible for its improvement in performance versus sodium butyrate, it is possible to design analogues which can perform as well as or better than panobinostat for LVV production.

Such analogues can also be studied for clinical indications and put forward for trials. Some analogues can even have additional therapeutic targets and have fewer side effects.

Panobinostat analogues have been produced by other groups previously and these analogues are extremely simple with zinc binding group (ZBG) substitution. One example includes HDAC-IN-27.

Synthistat Analogues

Our patent pending analogues incorporate further changes beyond ZBG substitution, we also use proprietary linker and cap groups

The resulting novel structures have better absorption characteristics and have improved molecular docking scores for various HDAC isoforms and thus have the potential for improved LVV production performance

Some of our candidates are also drug like molecules, which follow Lipinski rule of 5, and could have further potential as clinical candidates. Specific combinations of our proprietary cap and linker with non hydroxamic ZBG also unlocks targeting of additional targets, such as BRD4, PARP1 and D2R.

Exploring Additional Targets for HDAC Inhibitors

Dual-Mechanism Epigenetic Inhibitors

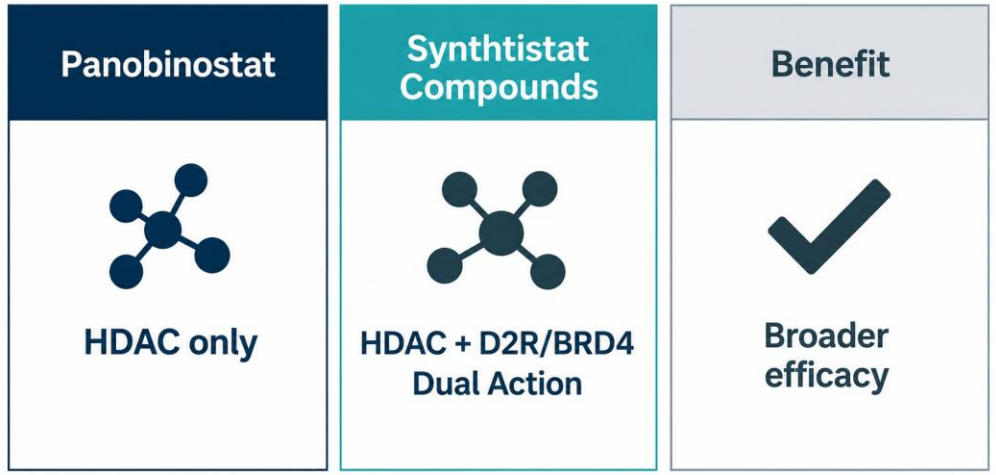
Select HDAC inhibitors, with proprietary cap and linker groups, exhibit **dual antagonism of HDAC isoforms and D2R or BRD4,** enabling targeting of both epigenetic regulation and survival or transcriptional pathways in **AML and solid cancers**.

Drug-Like Optimisation

Multi target lead compounds retain **favourable physicochemical and ADME characteristics**, outperforming benchmark HDAC inhibitors including panobinostat.

Potent Multi-Target Engagement

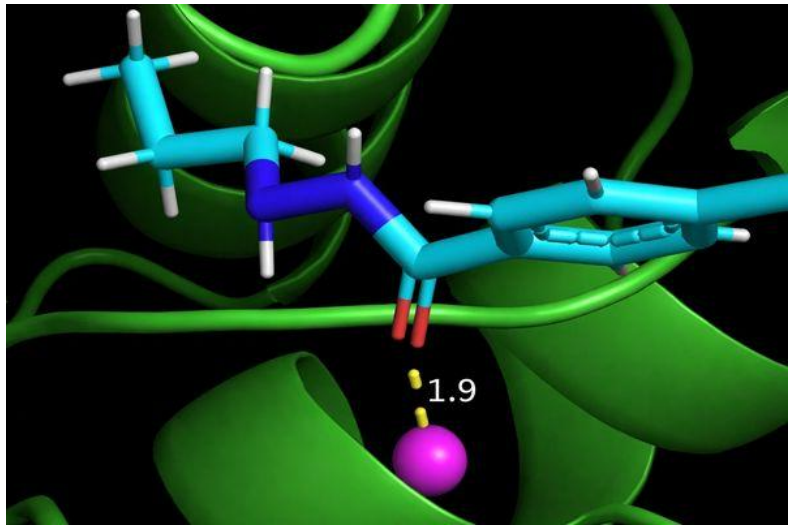
Predicted **nanomolar potency** across **HDAC2/6 and D2R or BRD4** supports differentiated single-agent activity.



Property	Mol7	Mol8	Pano
BRD4 (kcal/mol)	-8.1 (~2.8–3.1 Å ND2–O)	-7.9 (~2.8–3.1 Å ND2–O)	—
HDAC2 (kcal/mol)	-8.6	-8.6	-8.4
HDAC6 (kcal/mol)	-6.5	-6.2	-6.2
PARP1 (kcal/mol)	-10.3	-9.6	-8.6
D2R (kcal/mol)	—	-10.1	—

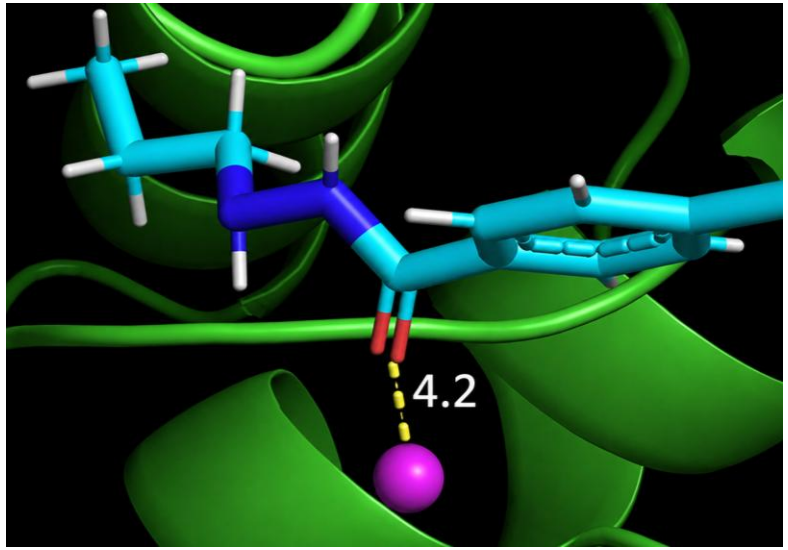
Ligand Pose Verification and Candidate Selection

- Candidate selection based solely on raw docking scores is highly inaccurate without ligand pose verification
- For classical HDAC inhibitor structures containing a cap, linker, and zinc-binding group, specific binding-geometry rules must be satisfied for a compound to be considered a credible hit
- For example, the ZBG of any HDAC inhibitor is expected to lie within coordinating distance of the catalytic zinc cation across target isoforms, typically around 1.9–2.8 Å
- Therefore, any candidate with a high docking score but without a validated ligand pose should be discarded as a false hit
- AutoDock Vina has also been reported to achieve over 80% accuracy in predicting binding poses for structures with 5–8 rotatable bonds. This is relevant because 95% of our library compounds with validated ligand poses have 8 or fewer rotatable bonds

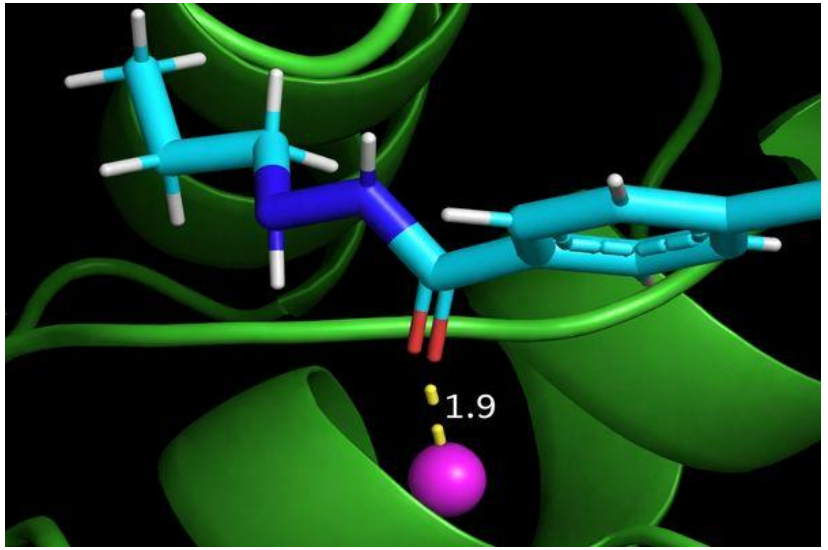


The carbonyl oxygen present within the hydrazide ZBG of this Synthistat HDACi candidate, is the correct distance from the zinc cation (1.9–2.8 Å). This is an essential element for a HDACi validated ligand pose. The docking score is -8.9.

Ligand Pose Verification and Candidate Selection

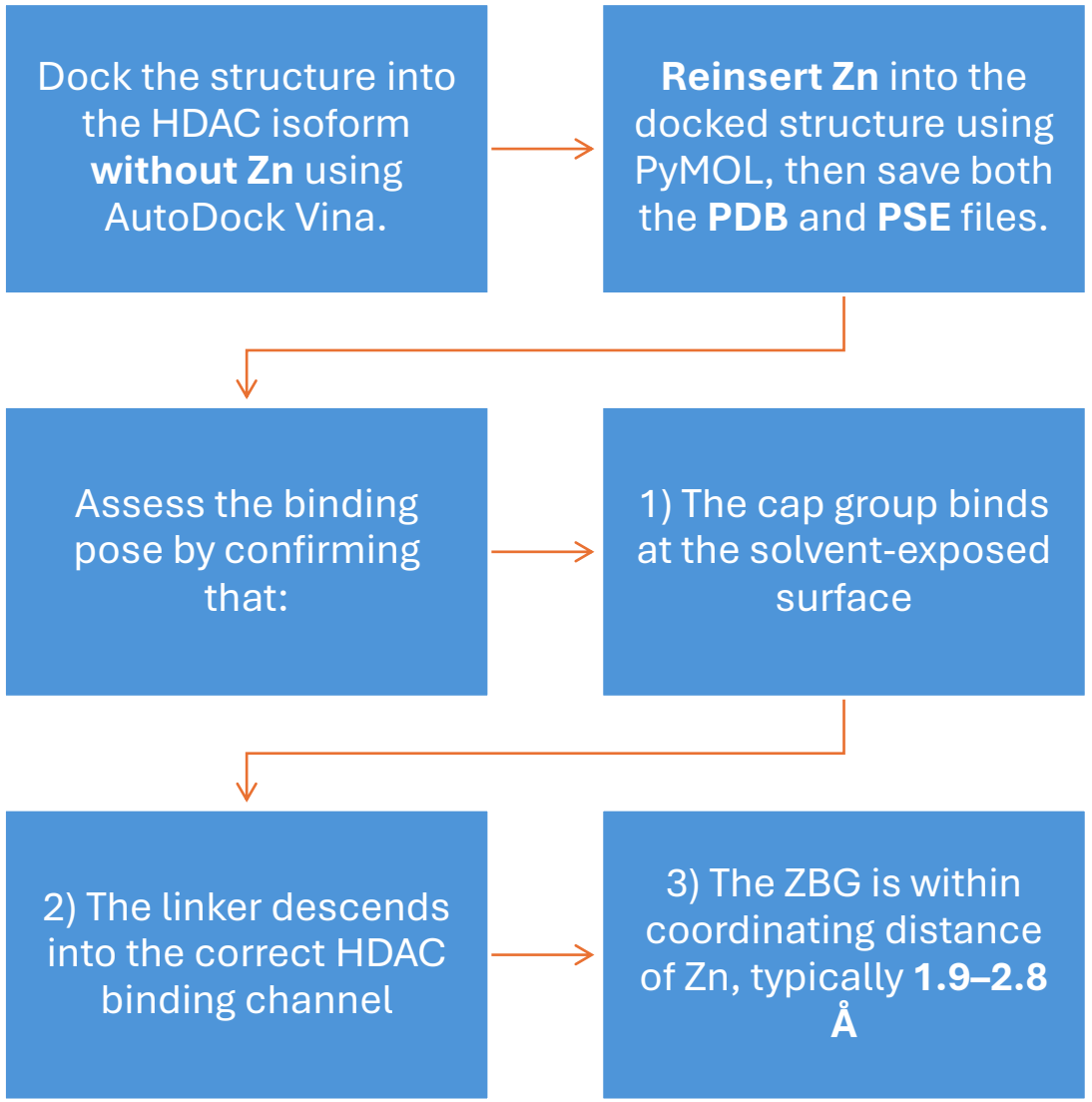


The carbonyl oxygen present within the hydrazide ZBG of this HDACi candidate, is not the correct distance from the zinc cation (1.9–2.8 Å) . Therefore, it would be discarded as a false positive hit, even with a docking score of -11



The carbonyl oxygen present within the hydrazide ZBG of this HDACi candidate, is the correct distance from the zinc cation (1.9–2.8 Å). This is an essential element for a HDACi validated ligand pose, the docking score is -8.9

Docking Work Flow



- Pose verification can be supported using publicly available AI tools such as ChatGPT by uploading the PDB file and requesting pose assessment. However, manual proof-checking is recommended to reduce the risk of hallucinated or incorrect interpretations.
- This workflow is effective for identifying structures in which the cap group and linker correctly orient the ZBG toward Zn-coordinating distance. It also has a lower technical barrier and can be performed using free software.

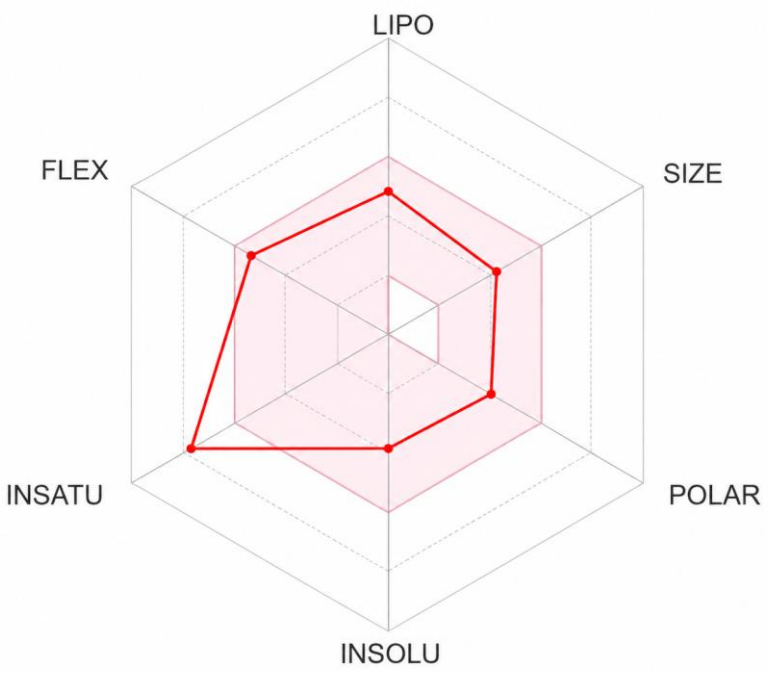
Physiochemical Cut-offs

- Any structure with a validated ligand pose but poor aqueous solubility or poor cellular absorption has limited practical value.

- Therefore, before undertaking exhaustive ligand pose verification, we apply a preliminary physicochemical selection filter to prioritize candidates that are more likely to be orally bioavailable and directly absorbed by cells in culture media.

- One suitable tool for this task is **SwissADME**.

- Primary cut-off criteria include:
  1) Predicted **high GI absorption**
  2) Compliance with **Lipinski's Rule of 5**
  3) Predicted **LogS above -4**



Improved Specificity

Panobinostat

RPMI-8226 Immunitoxicity	0.399	●	ⓘ
A549 Cytotoxicity	0.018	●	ⓘ
Hek293 Cytotoxicity	0.875	●	ⓘ
Reactive compounds	0.002	●	ⓘ
Promiscuous compounds	0.939	●	ⓘ

Synthistat Proprietary Analogue

RPMI-8226 Immunitoxicity	0.401	●	ⓘ
A549 Cytotoxicity	0.001	●	ⓘ
Hek293 Cytotoxicity	0.12	●	ⓘ
Reactive compounds	0.001	●	ⓘ
Promiscuous compounds	0.005	●	ⓘ

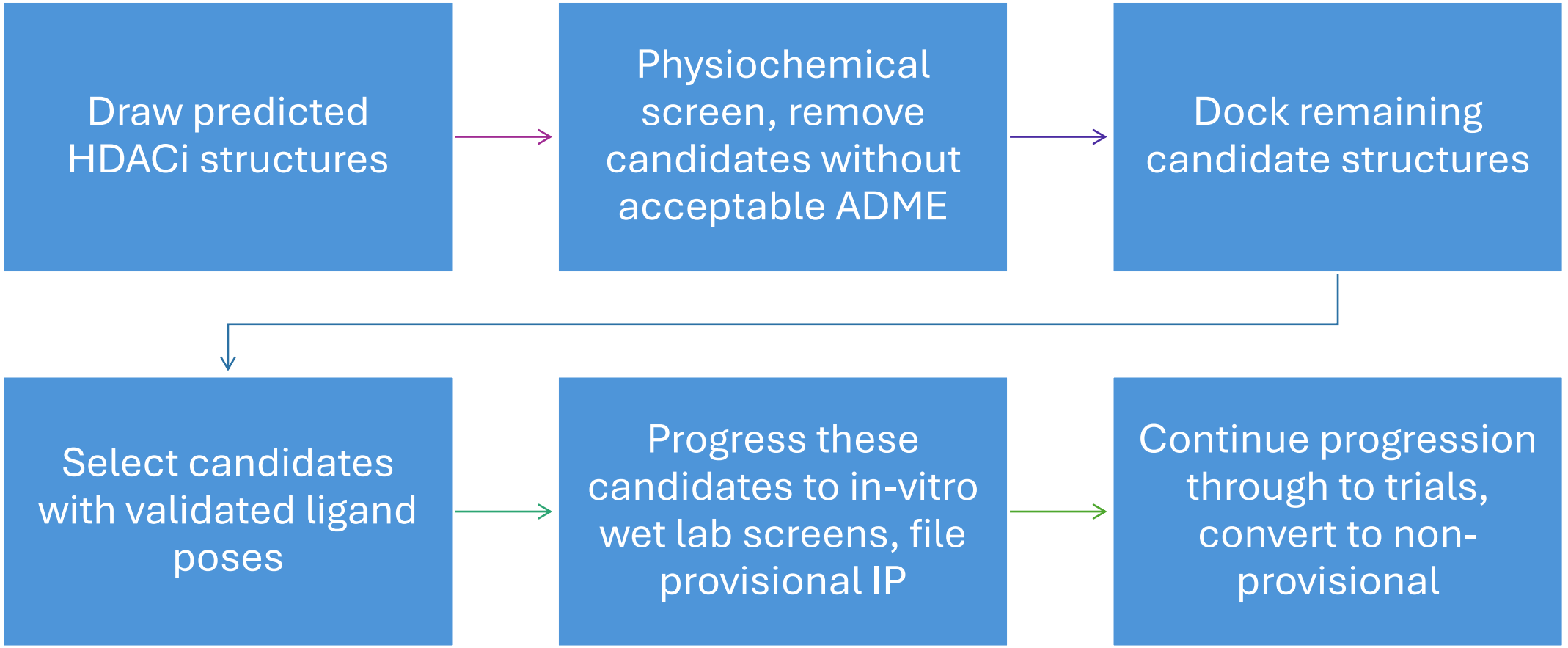
- Published non-hydroxamate HDAC inhibitors employing hydrazide or benzamide zinc-binding groups (ZBGs) have also been reported to retain potent HDAC inhibition while exhibiting reduced HEK293 toxicity relative to hydroxamate HDAC inhibitors, a trend also predicted by ADMETlab 3.0. Our proprietary hydrazide analogues similarly demonstrate substantially lower predicted HEK293 cytotoxicity than panobinostat, while maintaining comparable or greater predicted activity/toxicity signatures in RPMI-derived leukemia models.

- This differential sensitivity suggests improved cellular selectivity, which may be advantageous both clinically and in viral-vector-enhancer applications, particularly as HEK293-derived cells are widely used for lentiviral packaging and production.

- Our compounds additionally show substantially lower predicted promiscuity, aggregation liability, and hERG risk relative to panobinostat, including less hazardous predicted hERG binding geometries, supporting the hypothesis of more specific target engagement and improved cardiac safety.

- However, our analogues differ from known non-hydroxamate HDAC inhibitors through the incorporation of proprietary linker and cap-group architectures that produce the mentioned less hazardous predicted hERG binding geometries, while also potentially enabling broader polypharmacology, including predicted activity toward **BRD4, D2R, and PARP1**.

Putting It All Together!

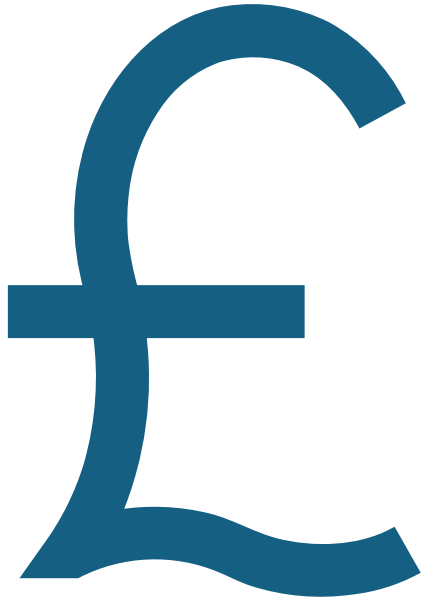


Potential Upside, LVV Enhancers

- The CDMO industry and research use viral vector industry is a multi-billion-pound market, at present CDMO companies must purchase new buildings and manufacturing equipment to increase capacity, with major upfront associated costs

- OXB for example had to fund 30 million pounds for their new manufacturing facility back in 2020 and this only resulted in a doubling of overall manufacturing capacity, while usage of a synthetic HDAC inhibitor would have significantly lower costs with concomitant increases of viral production of 2-fold or greater

- Synthetic HDAC inhibitors are also a cost-effective solution as a litre of virus production medium can be marketed for between £5,000-20,000, while research grade panobinostat for example only costs £10 a litre of virus production medium, while GMP grade can cost £148 per litre



Potential Upside, LVV Enhancers

- Taking this into consideration, we would not be charging clients the raw material cost of synthetic HDAC inhibitors, rather we would be charging a license fee, priced at a set percentage of revenue, say 5%

- Alternatively, we could be licensing the usage of our synthetic HDAC inhibitors within viral production kits which are marketed by companies like Thermofisher and Mirus, and we would again be charging a set percentage of sales revenue

Potential Upside, Clinical Drugs

- Panobinostat alone is disclosed to have a clinical market size of over a billion pounds a year, this is before we consider the market size of other approved HDAC inhibitors like vorinostat and chidamide

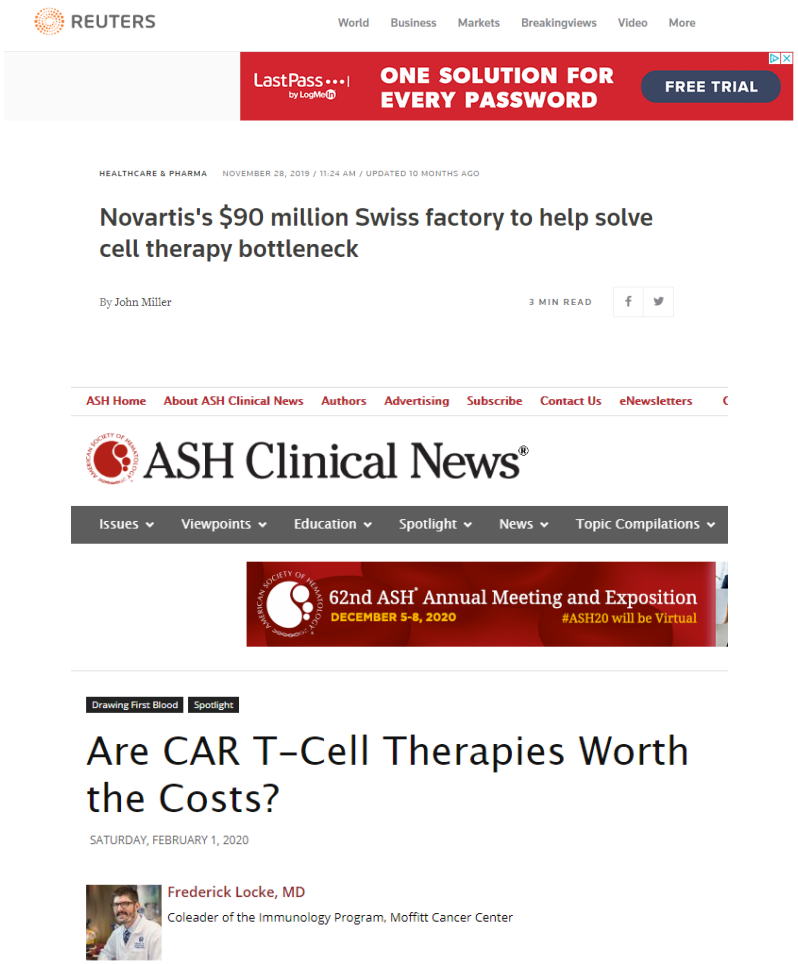
- Any synthetic HDAC inhibitors we produce, which are successful in clinical trials for any major clinical indication, like Acute Myeloid Leukaemia (AML), would similarly command massive valuations, certainly in the millions and possibly into the billions

- Income we receive from licensing these synthetic HDAC inhibitors as LVV production enhancers, could also be re-invested to support candidates into clinical trials. Alternatively, we may partner up with larger pharmaceutical companies to fund clinical trials and drug development

Project 2:

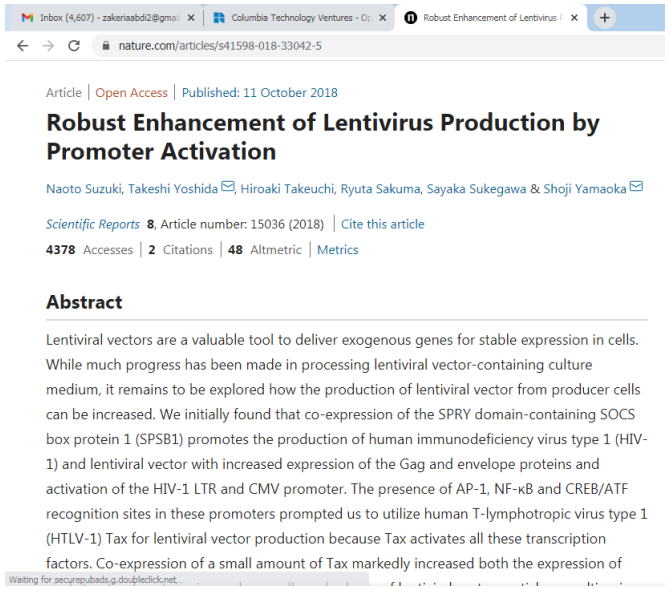
Gene Modified Producer Cell Lines for Gene Therapy Manufacturing

Synthistat and Gene Modified (Knock Out) Cell Lines



- Modified cell lines for augmented lentiviral vector production can help tackle the gene therapy bottle neck that is causing supply and affordability issues, by helping to increase the industries manufacturing capacity

- Genetic modifications that include knock ins, and knockouts, for viral vector production can be patented and commercialized. T-cell antigen knock in cell lines were patented by Stanford University, in 1998, Stanford Docket S97-079. HO-2 knockout/knockdown cell lines were patented by Columbia University, in 2016, WO2017210337A1



Inbox (4,607) - zakeriaabdi2@gmail | Columbia Technology Ventures - Op | Robust Enhancement of Lentivirus P

nature.com/articles/s41598-018-33042-5

Article | Open Access | Published: 11 October 2018

Robust Enhancement of Lentivirus Production by Promoter Activation

Naoto Suzuki, Takeshi Yoshida, Hiroaki Takeuchi, Ryuta Sakuma, Sayaka Sukegawa & Shoji Yamaoka

Scientific Reports **8**, Article number: 15036 (2018) | Cite this article

4378 Accesses | **2** Citations | **48** Altmetric | Metrics

Abstract

Lentiviral vectors are a valuable tool to deliver exogenous genes for stable expression in cells. While much progress has been made in processing lentiviral vector-containing culture medium, it remains to be explored how the production of lentiviral vector from producer cells can be increased. We initially found that co-expression of the SPRY domain-containing SOCS box protein 1 (SPSB1) promotes the production of human immunodeficiency virus type 1 (HIV-1) and lentiviral vector with increased expression of the Gag and envelope proteins and activation of the HIV-1 LTR and CMV promoter. The presence of AP-1, NF-κB and CREB/ATF recognition sites in these promoters prompted us to utilize human T-lymphotropic virus type 1 (HTLV-1) Tax for lentiviral vector production because Tax activates all these transcription factors. Co-expression of a small amount of Tax markedly increased both the expression of

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keriaabdi2@gmail | Columbia Technology Ventures - Op

Not secure | innovation.columbia.edu/technologies/CU16034_heme-oxygenase-2-inhibition-to

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Heme oxygenase-2 inhibition to increase lentiviral production efficiency

This technology is a method of increasing lentiviral production rates by inhibiting heme oxygenase-2.

Unmet Need: Large-scale, high-throughput production of lentiviral particles

Lentiviruses, a subfamily of retroviruses, are a powerful tool in gene editing technology. These potent viruses are able to infect both replicating and non-replicating cells, encoding viral RNA into the host cell's genome which results in sustained expression. Lentiviruses have also been a successful vector for the treatment of genetic diseases as well as hematopoietic stem cell therapy. However, large-scale lentiviral application is limited by high production costs and time requirements, as current manufacturing processes yield low and unreliable titers.

The Technology: Increased lentiviral production efficiency through heme oxygenase inhibition

This technology increases the efficiency of lentiviral production through inhibition of the cellular protein heme oxygenase-2 (HO-2). HO-2 slows the maturation process of viral particles by binding Gag, a major viral protein, and preventing delivery to the plasma membrane. Consequently, lowering HO-2 levels through either genetic

HIGHLIGHTS

Inventors

Stephen P Goff
Liang Tong
Yiping Zhu
Shukun Luo

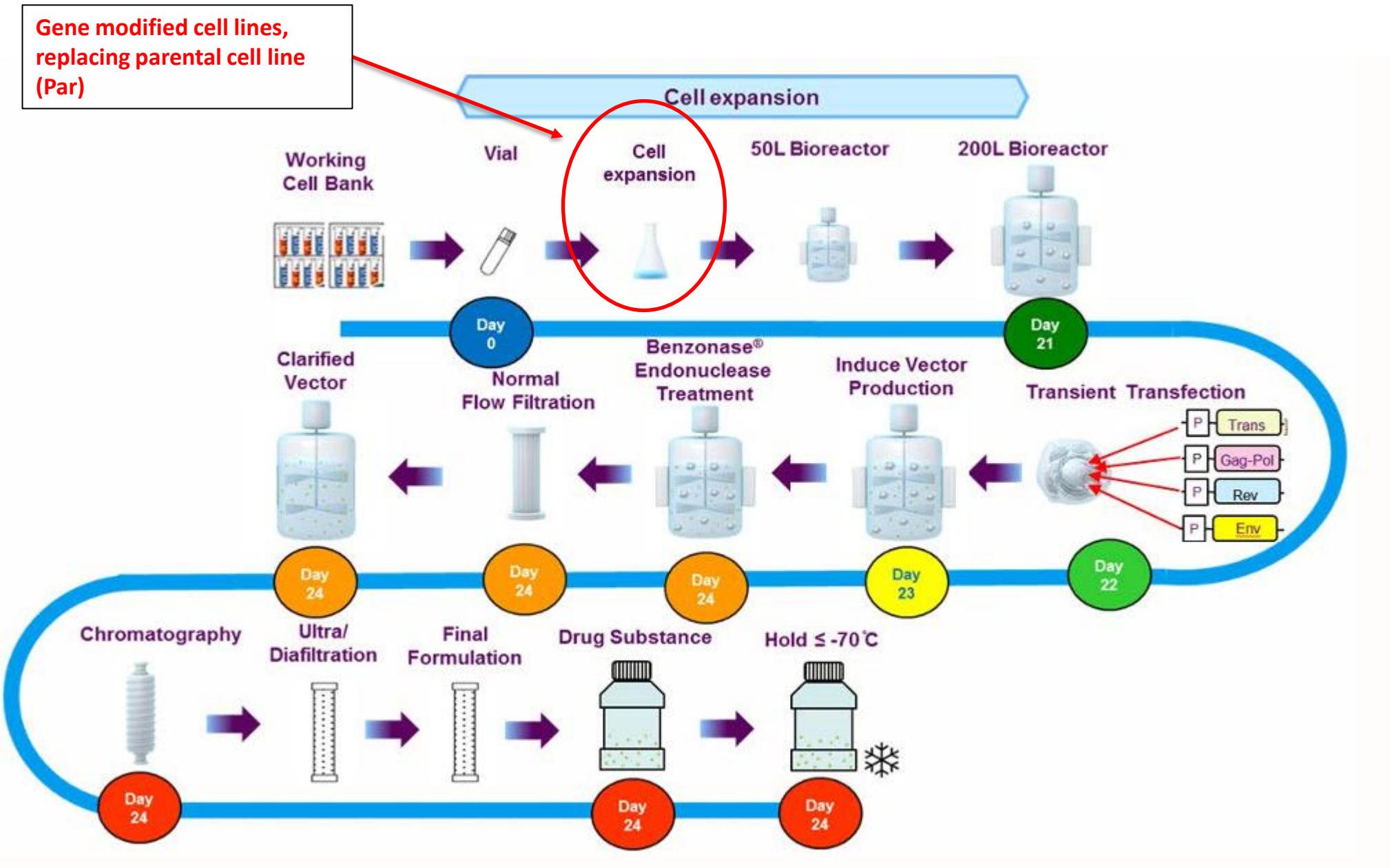
Tags

HMOX2
Lentivirus
Retrovirus
Gene Therapy
Virus
DNA
Genome
Titer

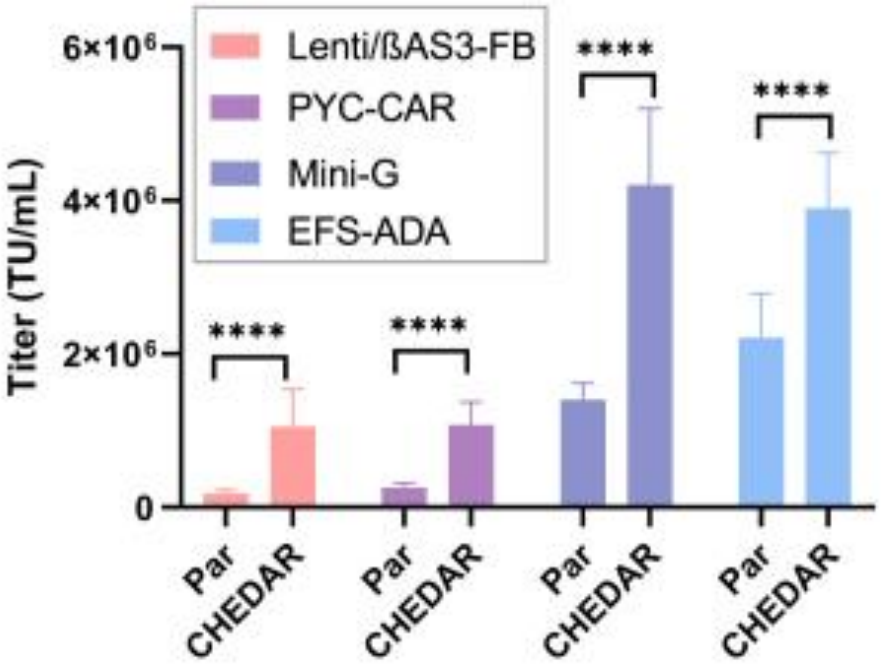
Background: Modified Lentiviral Vector Producer Lines

- Synthistat plans to screen, and commercialize, 29 knockdowns/knockouts and 26 knock ins, that support lentiviral vector biogenesis from producer cells. While identifying these targets, we also considered their impact upon cell viability and growth

- We hope to supersede HEK293T and take a market share in the multi-billion pound producer line industry and potentially levy license fees against contract manufacturing services


Gene modified cell lines, replacing parental cell line (Par)
Cell expansion
Working Cell Bank
Vial
Cell expansion
50L Bioreactor
200L Bioreactor
Day 0
Day 21
Clarified Vector
Normal Flow Filtration
Benzonase® Endonuclease Treatment
Induce Vector Production
Transient Transfection
P
Trans
P
Gag-Pol
P
Rev
P
Env
Day 24
Day 24
Day 24
Day 23
Day 22
Chromatography
Ultra/ Diafiltration
Final Formulation
Drug Substance
Hold ≤ -70°C
Day 24
Day 24
Day 24

Example Knockout Cell lines



- Figure from Han et al 2021, demonstrates how the CHEDAR cell line outperforms normal parental (Par) unmodified cell lines for 4 types of lentiviral vectors by up to 2-fold.

Source: Han, J., Tam, K., Tam, C., Hollis, R. P., & Kohn, D. B. (2021). Improved lentiviral vector titers from a multi-gene knockout packaging line. *Molecular therapy oncolytics*, *23*, 582–592. https://doi.org/10.1016/j.omto.2021.11.012

Synthistat Knockout Targets

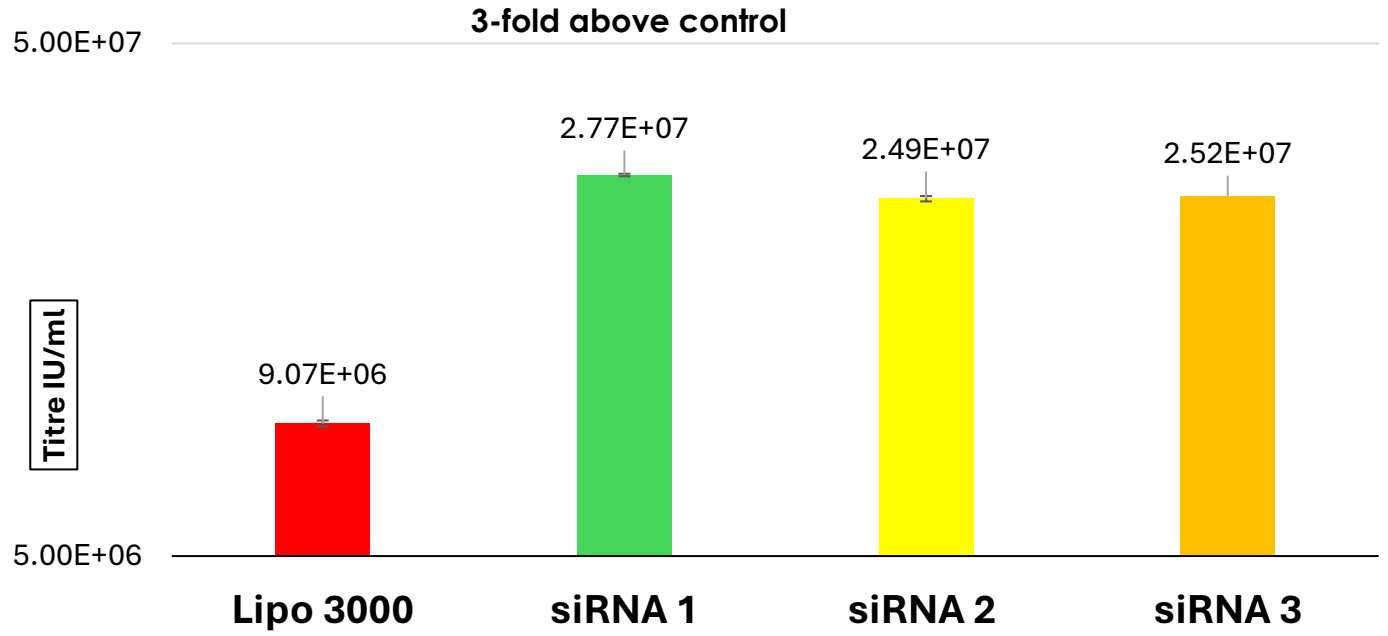
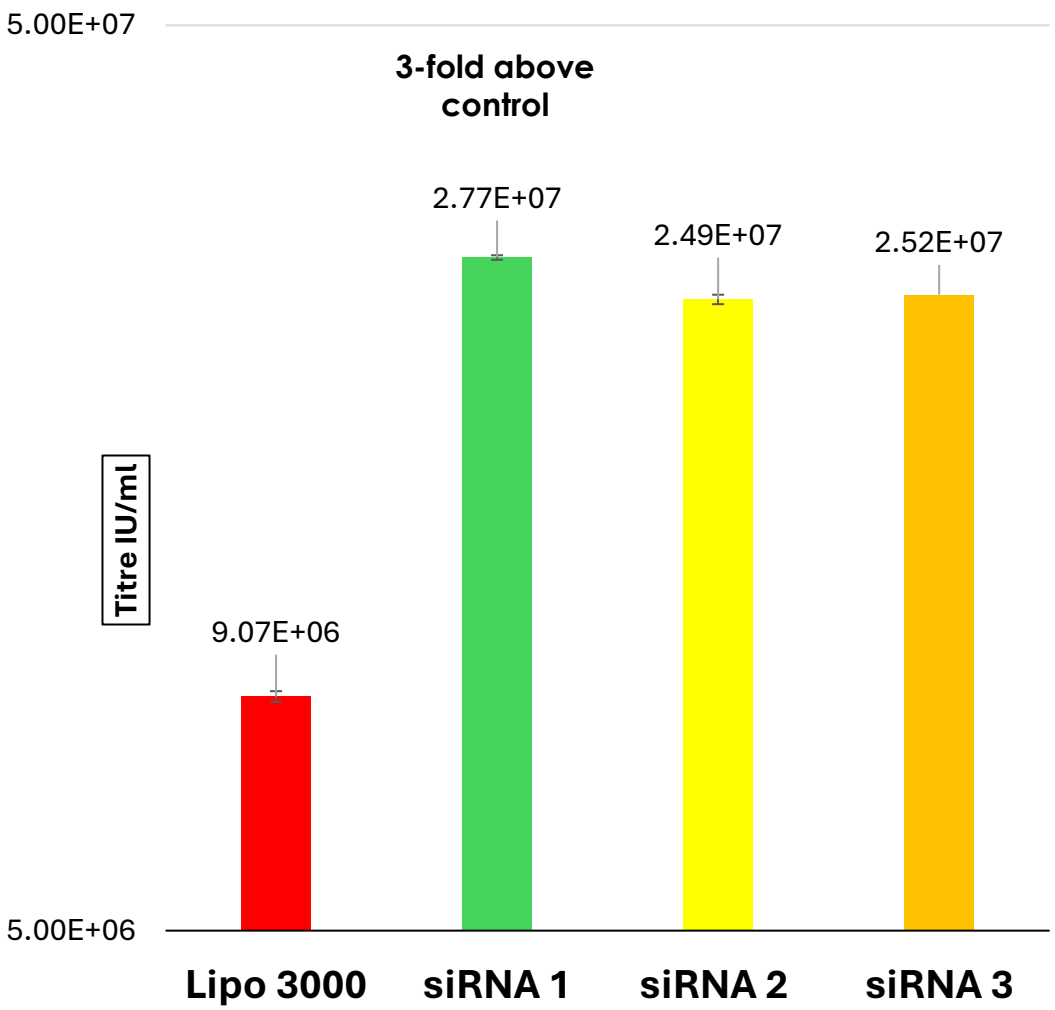


Figure 2, illustrates unpublished work, three gene targets were identified which could be transiently knocked down using siRNA, they can form the basis of a new knockout cell line, with similar performance to Han et al 2021, CHEDAR cell line. Further details can be found after scheduling a meeting with Synthistat Ltd (info@synthistat.com).



Available Immediately

Our proprietary siRNA titre enhancer technology for improving lentiviral vector production 2-3 fold, is also currently available for licensing.

Our protocol is also GMP compliant and validated for usage with clinically relevant CAR-T vectors.

Gene Modified Producer Line Advantages

1

Rapid increase in production capacity for CDMO clients, without high equipment costs or staff training

2

Easy to implement into manufacturing processes by simply swapping out unmodified cells and can be combined with synthetic HDAC inhibitors

3

No upfront costs for clients, they only pay based on what they earn, which should improve adoption!

Value Proposition Statement

Our gene modified producer lines can help biotech companies and universities, to generate lentiviral vectors more efficiently, offering time and cost savings that can not be offered by using other producer lines, such as parental HEK293T.

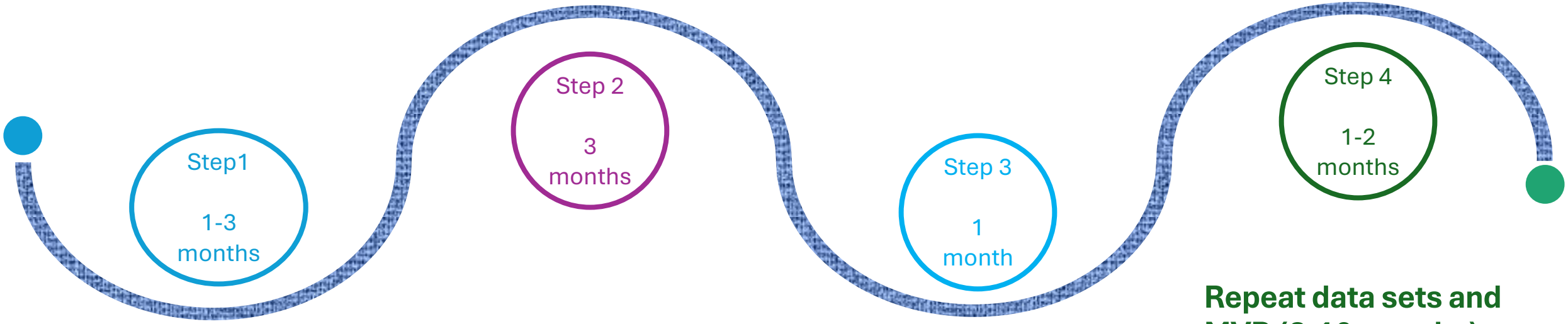
Provisional Patents Applied

We have already applied for provisional patents to protect the commercial interest in our projects!

Project 1: We have filed provisional patents on computationally screened, novel synthetic HDACi structures, with verified ligand poses, improved molecular docking scores, additional therapeutic targets and ideal ADME.

Project 2: In addition, we have filed upon 31 knockout and knockdown targets in HEK293 derived cell lines, which can be modulated to improve viral vector production.

ROAD MAP, PART I



Startup Phase

Provisional patents covering commercial project interest have already been applied for.

Securing suitable lab space, ordering and organising materials and reagents.

Office hours can be used to support product design and review.

Commencing Lab Work (4-6 months)

After organising the lab and becoming orientated, we should be heading towards commencing experimental work.

There is also expected lead time on ordering of synthetic HDAC inhibitors and knockout cell lines (12 weeks), however virus production runs should take place in the lab to be fully prepared and to test out all the equipment.

First data sets (5-7 months)

At this stage the lab should have been open at least 12 weeks, everyone should be fully orientated and the first data sets on synthetic HDAC inhibitors and knockout cell lines should have been generated.

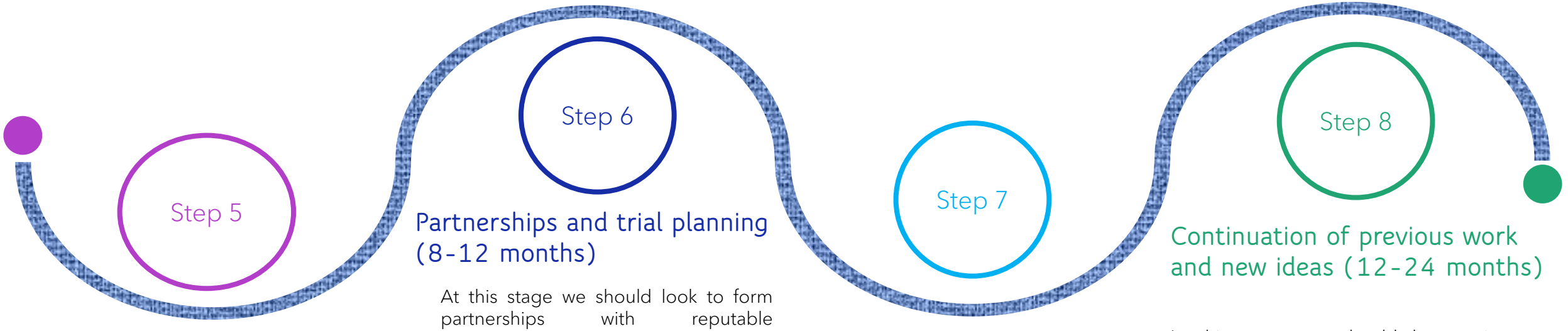
Repeat data sets and MVP (8-10 months)

After collecting initial data, we should have some repeat studies completed to N of 3 and have some minimal viable products.

We should ideally have found a novel synthetic HDAC inhibitor which performs as well as or better than panobinostat for lentiviral vector production.

We should also have identified some knockouts that improve lentiviral vector production.

ROAD MAP, PART II



Publications and Marketing (8-12 months)

Upon developing the first MVP, we should seek to publish in peer reviewed journals and present at international conferences like the ISCT.

We should also seek to contact prospective clients for product demonstrations.

Partnerships and trial planning (8-12 months)

At this stage we should look to form partnerships with reputable pharmaceutical companies and funding bodies like the Catapult, to support the interest of forwarding our synthetic HDAC inhibitors for pre-clinical and clinical studies.

We should also be looking at getting regulatory approval for our knockout cell lines, but this should be relatively straight forward and not costly.

First Revenues (12-24 months)

There is the potential to gather license fees from the research use and pre-clinical LV production market at this stage.

Following regulatory approval for either our HDAC inhibitors or knockout cell lines, we should be able to begin offering our technologies to clients for license fees.

Continuation of previous work and new ideas (12-24 months)

At this stage we should be an income generating company and we may continue developing HDAC inhibitors for usage as LVV titre enhancers and clinical candidates, we may also explore the space of isoform specific HDAC inhibitors, which is more focused on clinical usage.

We can also continue development of our gene modified cell lines or take steps to develop and formulate new business ideas.

We may also consider take over interests as a route to expanding the business and getting new ideas. The directors may also consider sale of the company.

Model License Fee Example

Oxford Biomedica, 2024, manufacturing revenue £68.4 million pounds

Synthetic HDAC Inhibitor, Production Enhancer, license fee 5% revenue

£68.4 million x 0.05 = **£3.24 million per annum**

Even a single CDMO client for a synthetic HDAC inhibitor license can garner very significant income! Likewise for a gene modified cell line license!

Contact

- Arfan Afzal, Synthistat, Non-Executive, Chairman
- Zakeria Abdi, Synthistat CEO and Lead Scientist
- MSc Pharmaceutical Sciences, Aston University, Distinction
- Email: info@synthistat.com

- Publications:

Abdi et al 2025, “Panobinostat for Improved LVV Production Versus Other HDAC Inhibitors”, Cytotherapy, 27 (5), available from: <https://www.sciencedirect.com/science/article/abs/pii/S146532492500146X>