Case Study: Developing liposomal formulation of a novel 91-mer synthetic peptide for treatment of ER+ CDK4i resistant Breast Cancer

CONCARLO THERAPEUTICS

TIDES 2023

Natalia Zisman

Disclaimer

This presentation contains proprietary information of Concarlo Therapeutics ("Concarlo") and is intended for discussion purposes, is not for further distribution, and should be treated in a confidential manner. It does not purport to be complete, and no representation or warranty, express or implied, is made by Concarlo, or any of its representatives as to the information contained in these materials or disclosed during any related presentations or discussions.

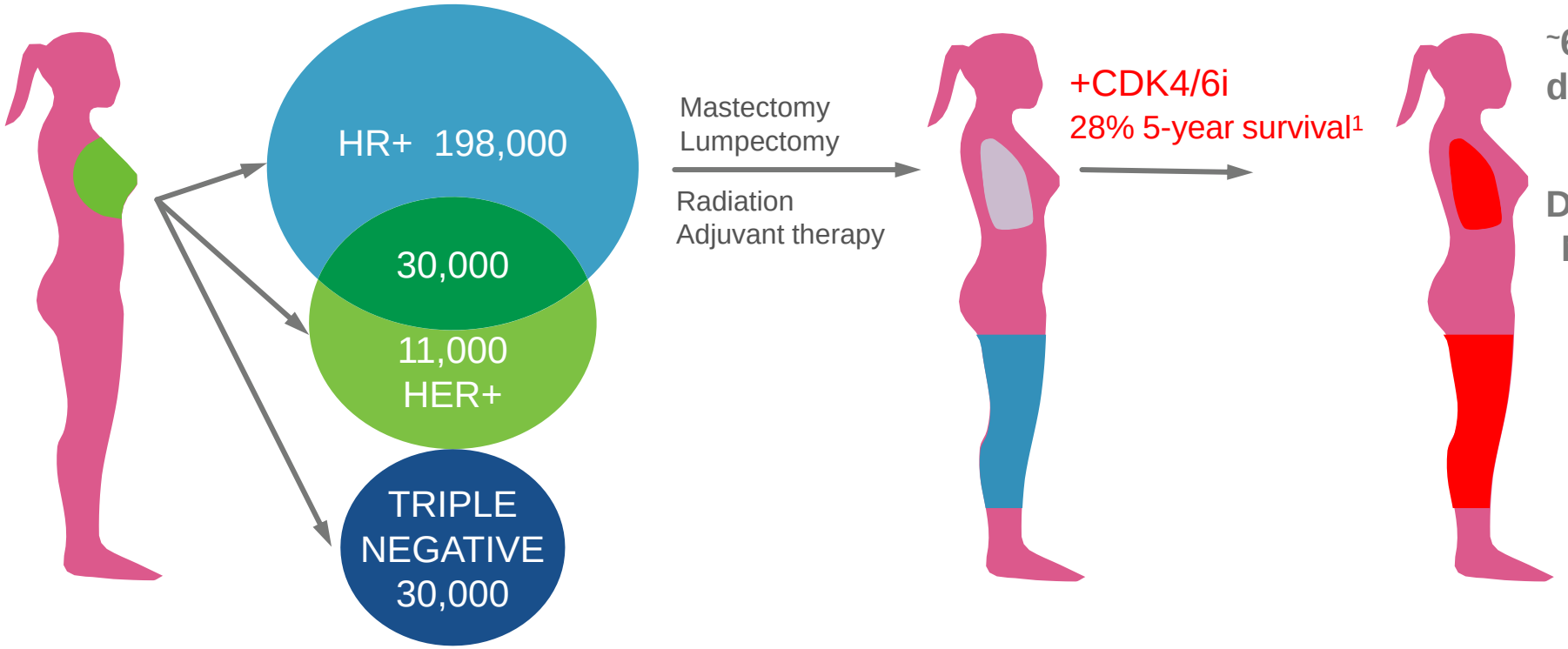
CONCARLO THERAPEUTICS

CDK4 inhibitors have added treatment arsenal of breast cancer, but resistance to these has become an urgent, unmet need

276,000 US women/yr
Develop breast cancer[1]

~43,000 HR+/HER2-
metastatic patients/yr[3]

~43,000
US deaths/yr



~660,000 WW
deaths/yr

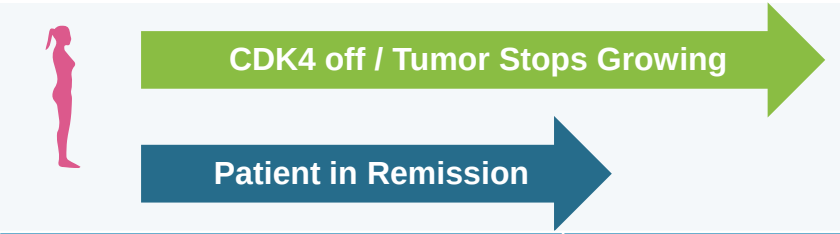
Drug Resistant
New therapies
Needed

1 NCI, Cancer Statistics Facts, accessed Feb 12, 2021
2 Company reported 2020 product revenue
3 GlobalData, HER2- Breast Cancer, Feb 2020

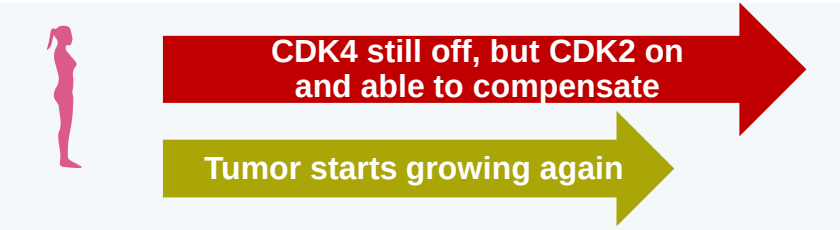
CONCARLO THERAPEUTICS

While targeting CDK4 has allowed for longer remission, nearly all patients eventually become resistant

Current CDK4 Inhibitors result in delayed progression



~ 6 Month Overall Survival benefit



SOLUTION:

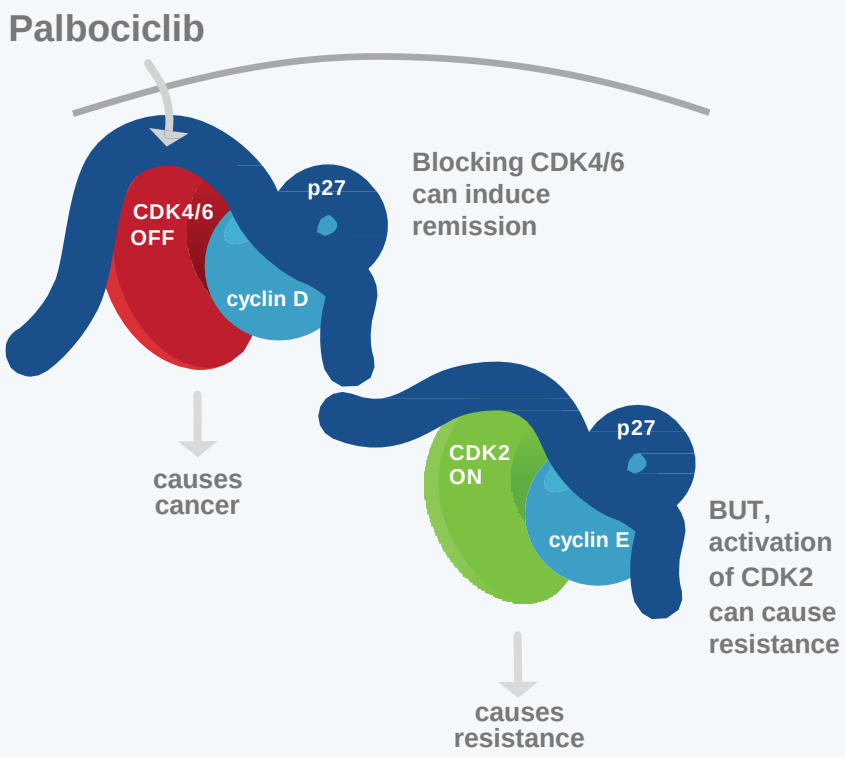
Need to drug CDK4/6 and CDK2 together to combat resistance

COMPANY / DRUG	TARGETS	STATUS	Serious Adverse Events
Tolero Pharma / Flavopiridol	CDK2/1/9/4/6/7	66 Ph1 and 2 trials, not registered for clinical use	DECREASES IN Neutrophils Platelets Red blood cells GI distress Eye toxicity
Merck & Co / Dinaciclib	CD2/5/1/9	18 Ph 1, 2, 3 trials, not registered for clinical use	
Syros / SY-5609	CDK7/2/9/12	Ph1	
Nuvation / Nuv-422	CDK2/4/6	Ph1/2 trial, Discontinued 8/2022	
Pfizer / PF-06873600	CDK2/4/6/1	Ph2 trials Discontinued 2/2023	
Blueprint Medicine / BLU-222	CDK2	Ph1/2a on hold by FDA due to SAE	
Incyclix / INX-315	CDK2	Pre-Clinical	

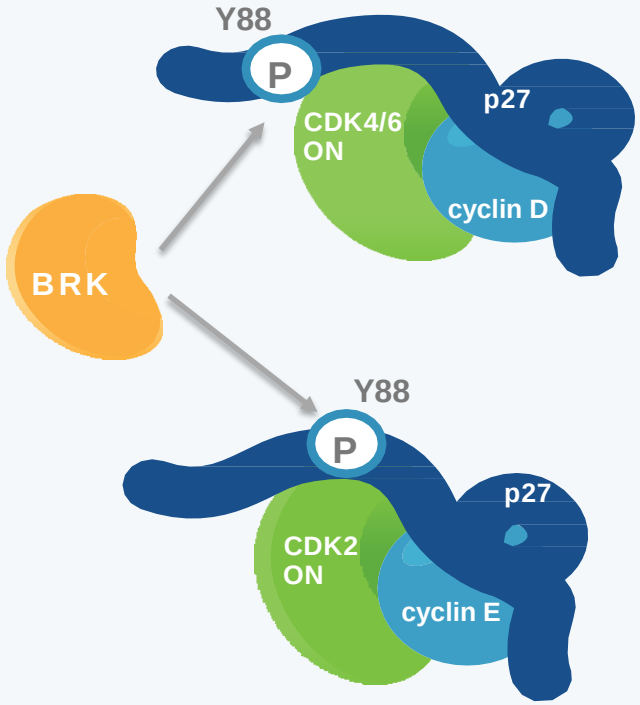
CONCARLO THERAPEUTICS

Concarlo Discovered a novel "ON-OFF" switch that controls CDK4/6 Activity: p27 Phosphorylation

Targeting only CDK4 causes resistance



Phosphorylation of p27 regulates both CDK4 and CDK2 activity



BRK (breast tumor related kinase) interacts with p27 to phosphorylate p27 on residue Y88, opening and activating the CDK4 and CDK2 complexes, driving proliferation

James, et al., Mol Cell Bio. 2008; Ray, Mol. Cell Bio. 2009; Hukkelhoven, J. Bio. Chem. 2012; Patel, et al. Mol. Cell Bio. 2015, Jilishitz, et al. Mol. Cancer Research. 2021, Mol Cancer Res

CONCARLO THERAPEUTICS

Publications

Published OnlineFirst January 12, 2018; DOI: 10.1158/1541-7786.MCR-17-0602

Cell Cycle and Senescence

Molecular Cancer Research

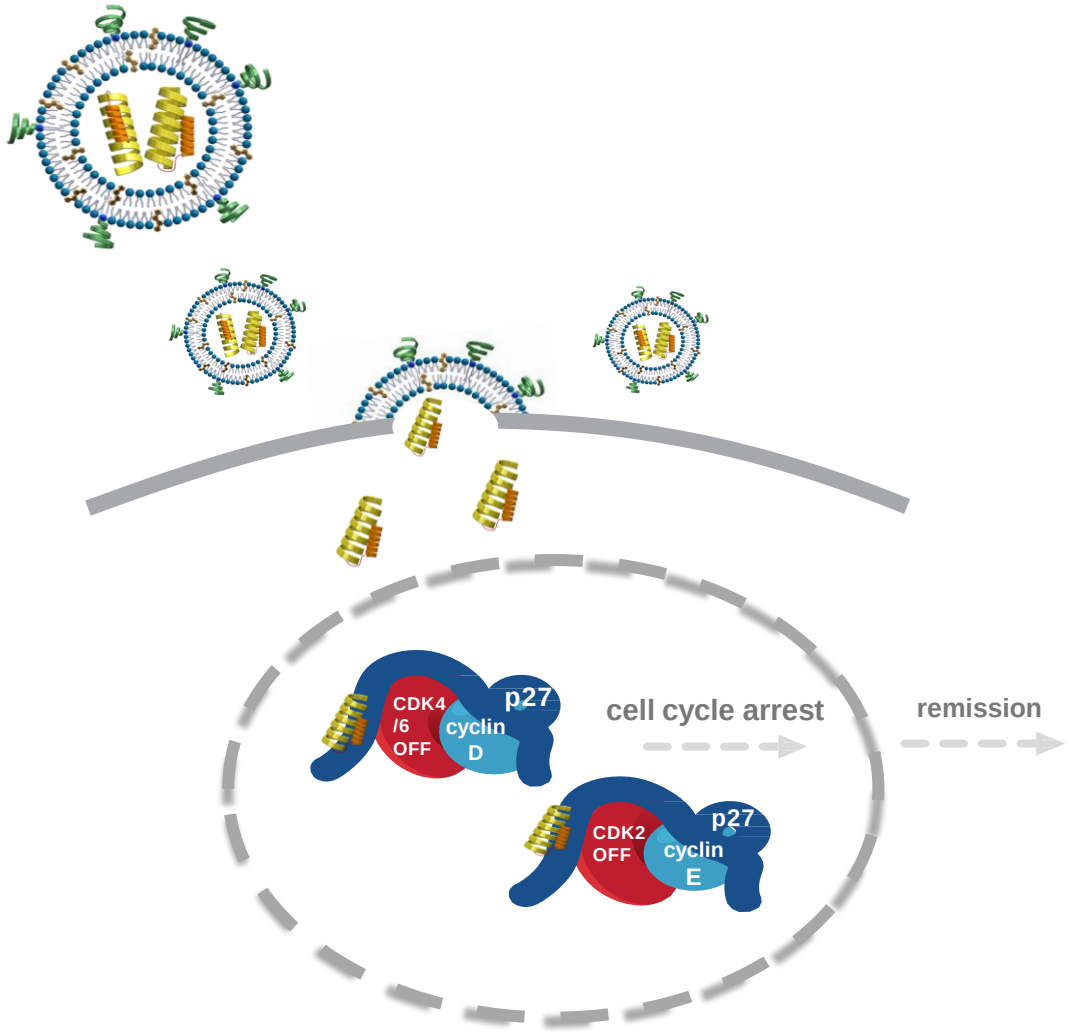
Dual Inhibition of CDK4 and CDK2 via Targeting p27 Tyrosine Phosphorylation Induces a Potent and Durable Response in Breast Cancer Cells

Priyank Patel[1], Vladislav Tsiperson[2], Susan R.S. Gottesman[3], Jonathan Somma[3], and Stacy W. Blain[2]

MOLECULAR CANCER RESEARCH | NEW HORIZONS IN CANCER BIOLOGY

NP-ALT, a Liposomal:Peptide Drug, Blocks p27Kip1 Phosphorylation to Induce Oxidative Stress, Necroptosis, and Regression in Therapy-Resistant Breast Cancer Cells

Irina Jilishitz[1], Jason Luis Quiñones[1], Priyank Patel[2], Grace Chen[2], Jared Pasetsky[3], Allison VanInwegen[1], Scott Schoninger[3], Manasi P. Jogalekar[1], Vladislav Tsiperson[1], Lingyue Yan[4], Yun Wu[4], Susan R.S. Gottesman[5], Jonathan Somma[6], and Stacy W. Blain[1]

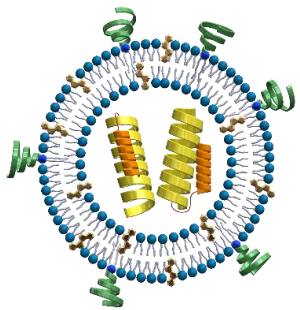


CONCARLO THERAPEUTICS

Concarlo targets CDK ON/OFF switch directly with IpY.20

CCL20: Novel Peptide Targeting p27

- CCL20 is a peptide based on **ALT**erntatively spliced form of BRK formulated in lipid nanoparticle IpY;
- Peptide has multiple potential interactions sites with p27, increasing specificity by:
  - successful binding and immobilizing the complex;
  - blocking of phosphorylation sites

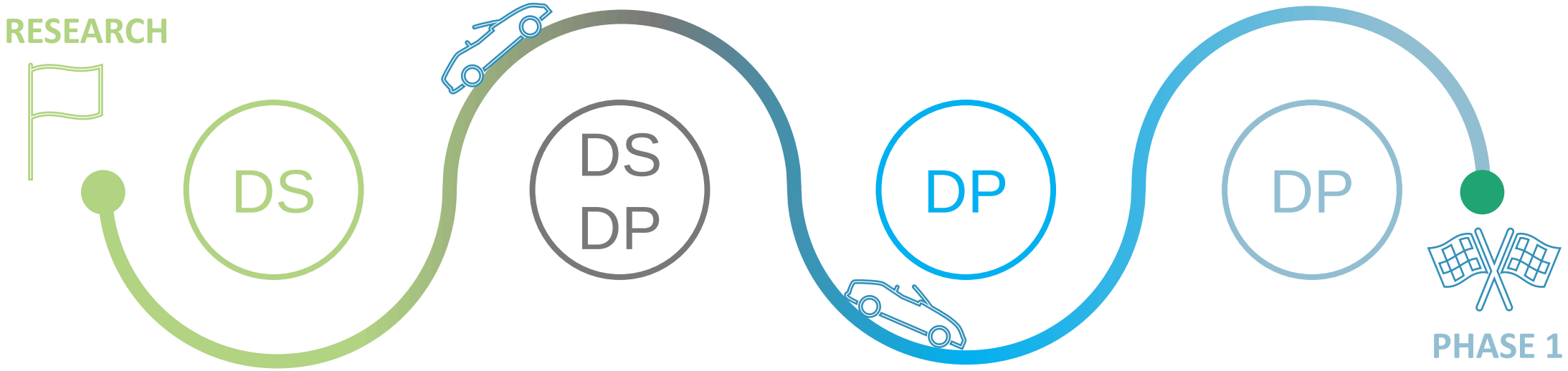


IpY.20: Liposomal Formulation of CCL20

- Lipid nanoparticles (LNPs) are safe and clinically proven delivery system for intercellular targets;
- LNPs formulated with fusogenic lipids can increase intercellular peptide delivery;
- Liposomes have been used in cancer treatment since 1995 (Doxil®). Currently 18 Liposomal Drug Product on the market;
- Liposomes can extend half life of peptide in plasma and accumulate at the tumor site;

CONCARLO THERAPEUTICS

Lab to Clinic: the long road of translation



PROPERTIES

- Solubility
- Salt Form Selection
- Purity

MANUFACTURING

- Complexity
- Reproducibility
- Scalability / Custom Equipment
- Production Costs

EVALUATION

- In vitro screening
- Preclinical PK / PD
- Preclinical Efficacy and Toxicology

CHARACTERIZATION

- Size / Charge
- Drug to Lipid Ratio
- Encapsulation Efficiency
- Morphology

CONCARLO THERAPEUTICS

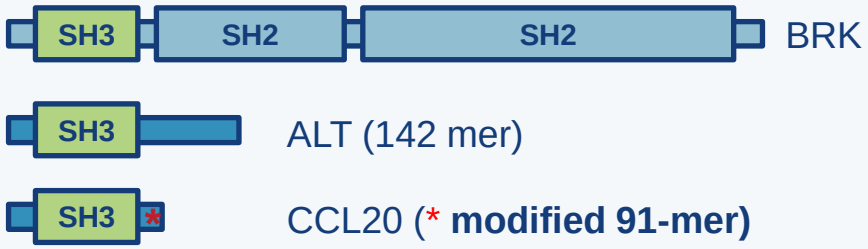
DS:Characteristics of Concarlo's Therapeutic Peptide

Nature knows best

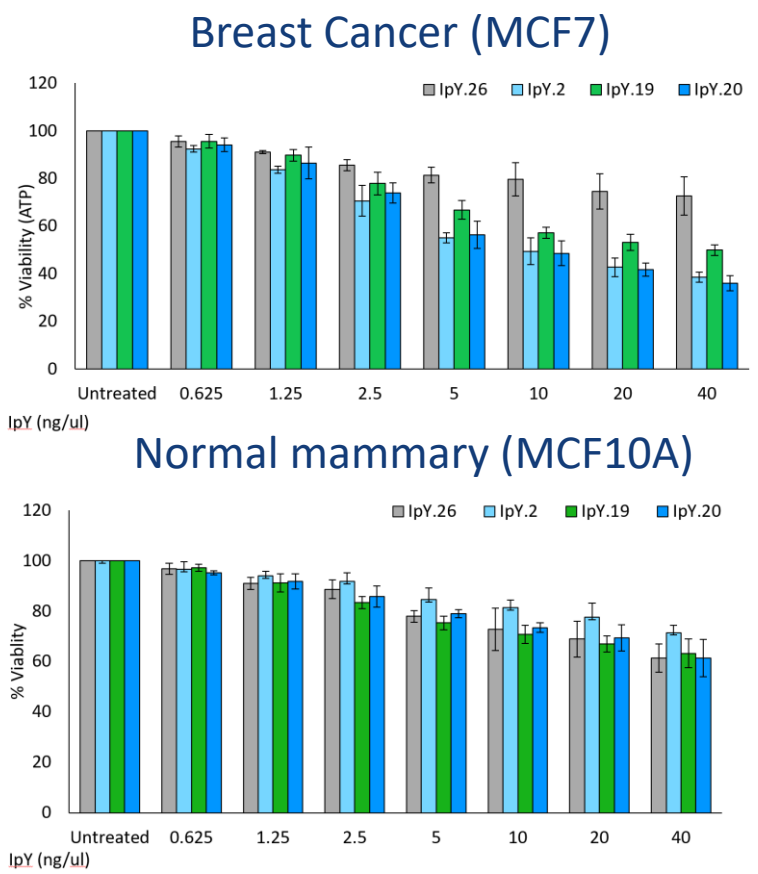
CCL20 is based on the naturally occurring ALTernatively spliced form of BRK

- BRK binds to p27 via its SH3 domain
- CCL20 is bioengineered to retains SH3 domain, with increased specificity and reduced toxicity
- CCL20 was derived from screening of multiple peptide variants in both ER/PR breast cancer cells and normal mammary breast cell lines

Protein Isoforms



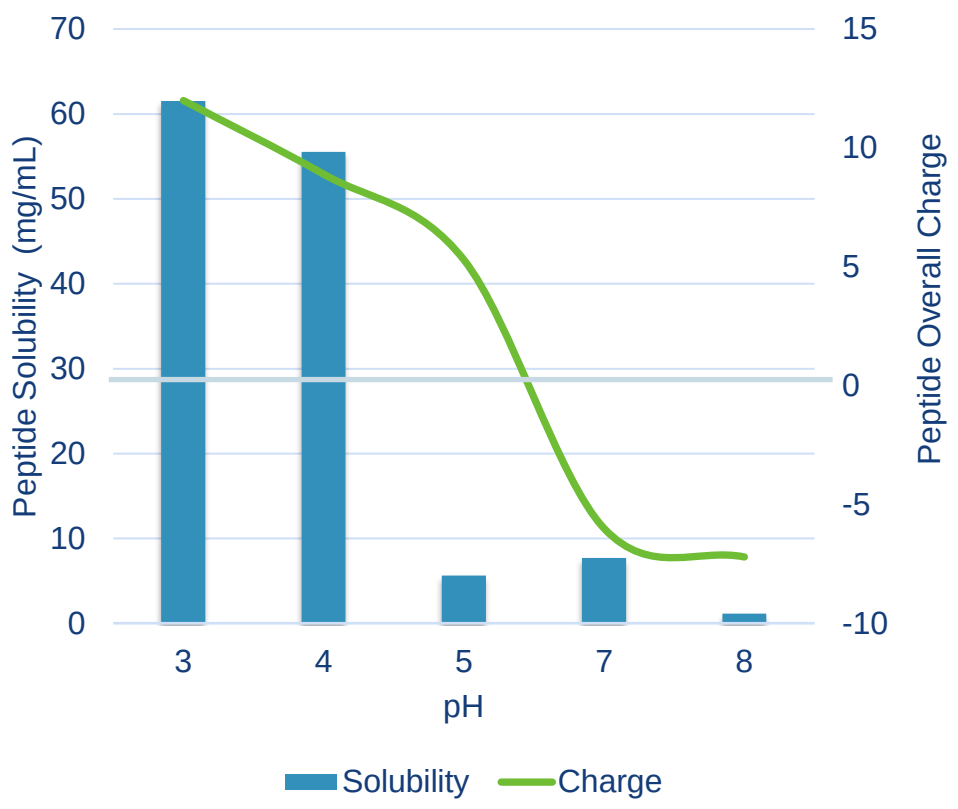
In vitro Screening in Breast Cancer (MCF7) and normal mammary cell lines (MCF10A)



CONCARLO THERAPEUTICS

DS: Systematic buffer screening approach significantly improved CCL20 solubility

Peptide Charge vs Solubility Relationship

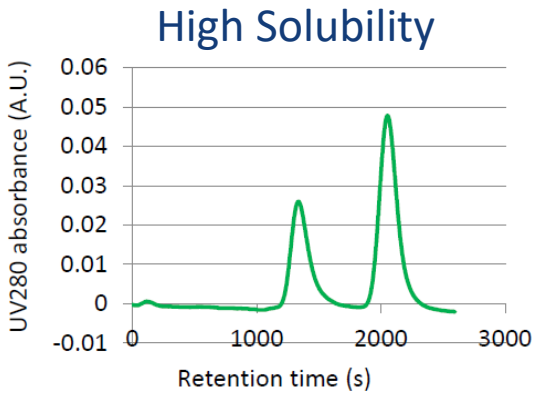


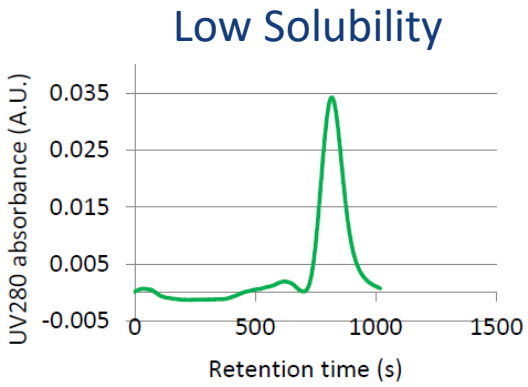
Improved Solubility and Conformation Stability

Rational design to solubility studies with minimal material requirements

Screened 30+ buffer components and compositions

Self-interaction chromatographic **(SIC)** experiments





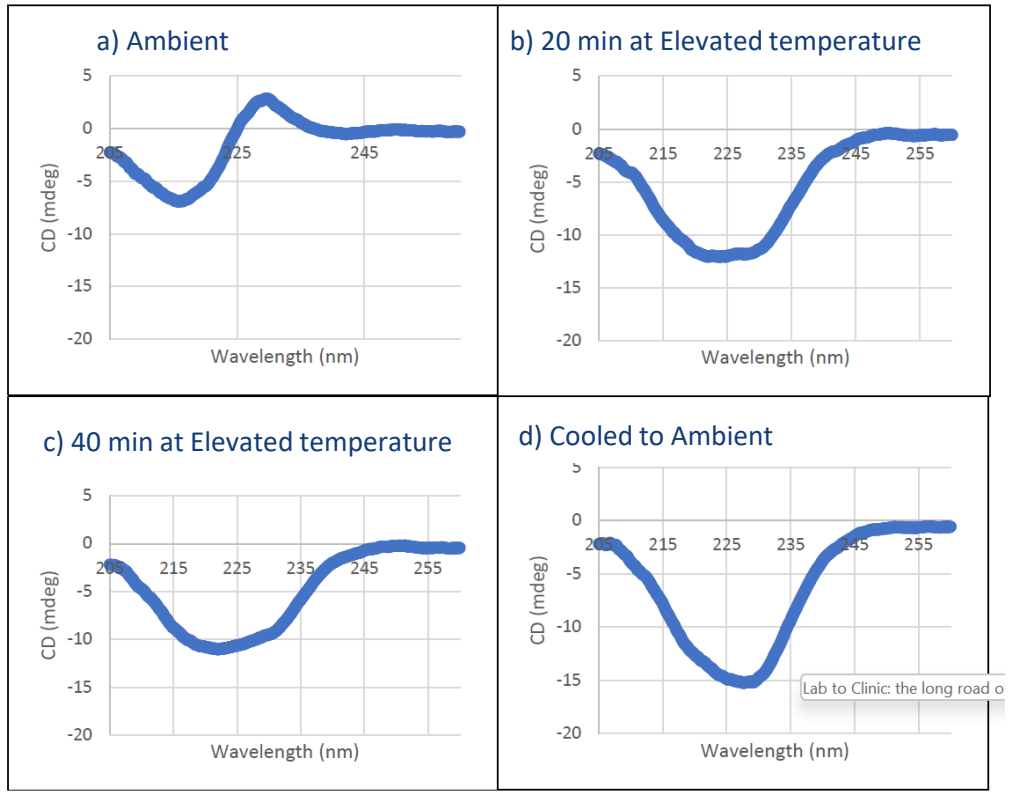
Peptide (Peak #1) eluted on SIC column in comparison to non interacting reference molecule (Peak #2) in different buffer conditions

Predictive Oncology

CONCARLO THERAPEUTICS

DS: Peptide Characterization Led to Insights on Peptide Thermal Stability

Peptide Conformation (CD) Affected By Temperature



Improved Solubility and Conformation Stability

Characterized Peptide aggregates by Dynamic Light Scattering **(DLS)** and Circular Dichroism **(CD)**

Evaluated the effect of temperature at peptide conformation stability

Identified multiple promising complex buffer compositions

Reduced solubilization time from ~2h (heating & sonication) to 15 mins (ambient, gentle mixing)

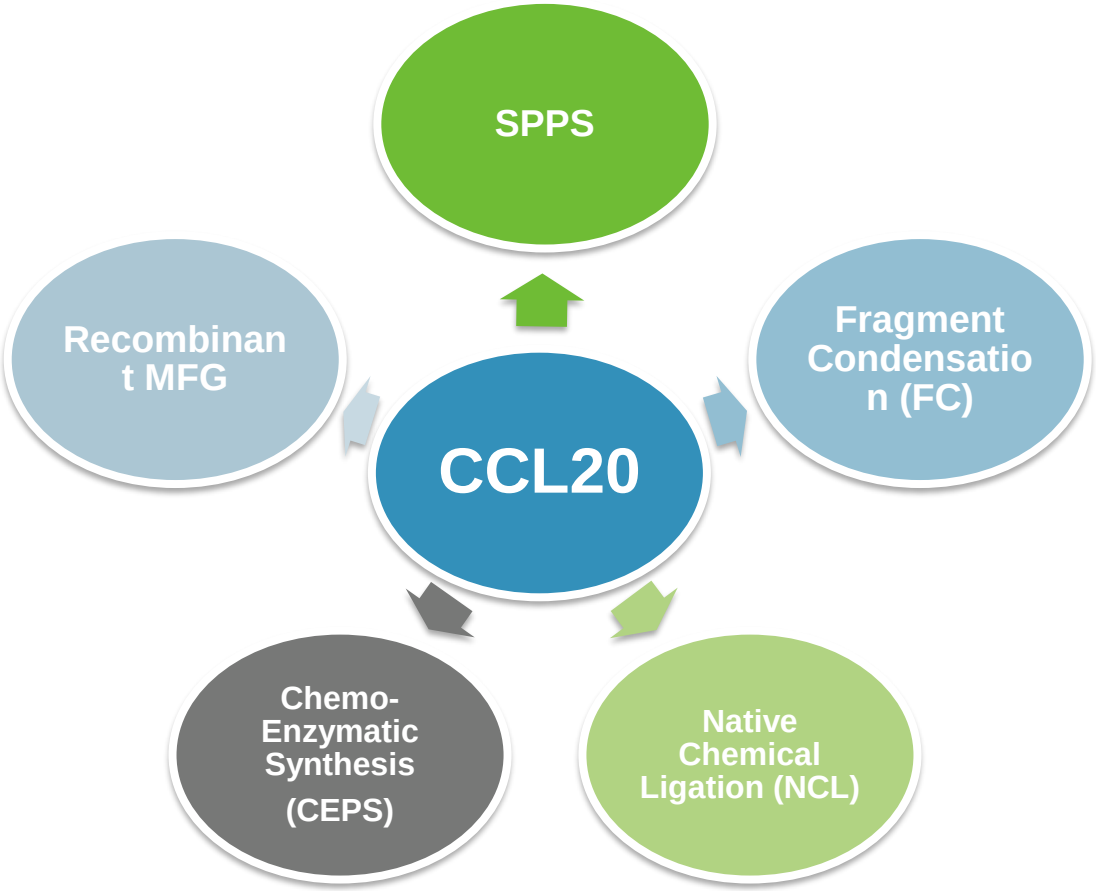
Predictive Oncology

CONCARLO THERAPEUTICS

DS: Choice of Peptide Manufacturing Route

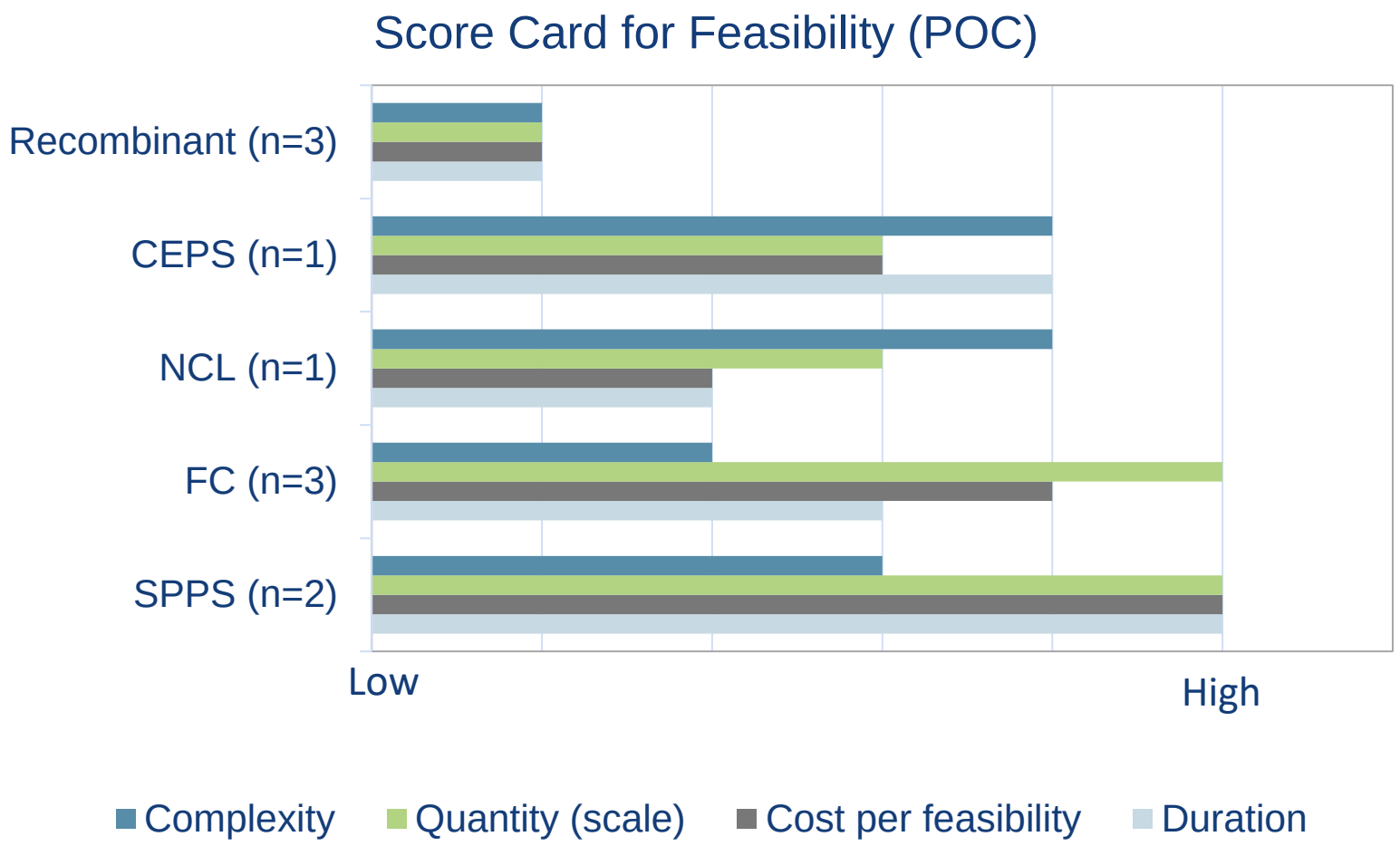
Peptide Manufacturing Challenges

- Yield
- Scalability
  - ➢ Hazardous solvents solvent / harsh chemistry requirements
- Purity
  - ➢ Impact of impurities on tolerability
- Analytical methodology
  - ➢ Importance of orthogonal methods of analysis
- Cost
- IP Landscape



CONCARLO THERAPEUTICS

DS: Factors for Selecting Manufacturing Route for CCL20



Additional Considerations for CMO Selection:

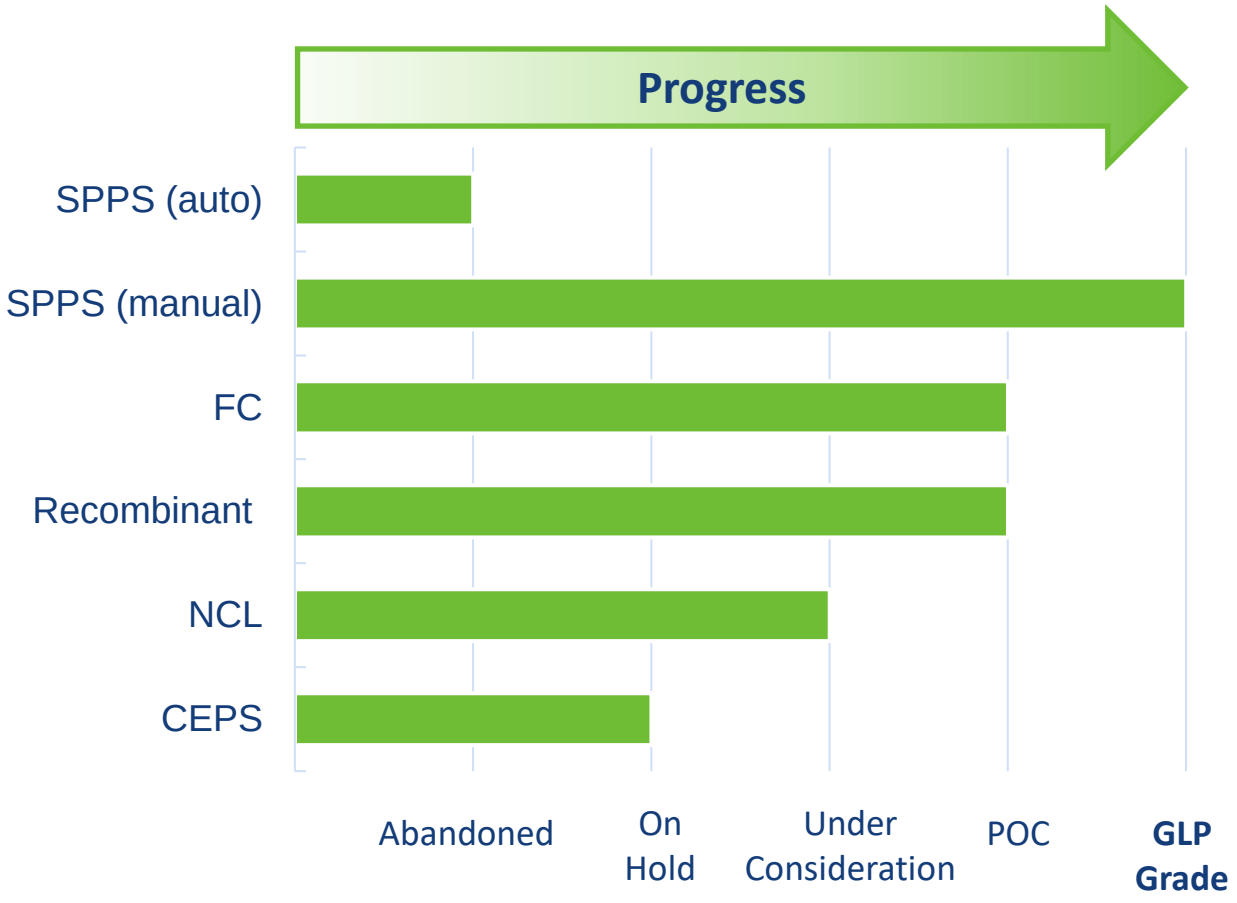
- IP
  - Supplier
  - Client
  - 3rd party
- Communication
  - Email only
  - Regular e-meetings
  - Ability to visit
- Supply Chain Logistics
  - Cross border
  - Cross continent

CONCARLO THERAPEUTICS

DS: Lessons learned from manufacturing of long peptides

Manufacturing

- Sterically challenging peptide regions contributed to long/incomplete coupling reactions in both SPPS and FC approaches;
- Purification process was challenging due to peptide hydrophobicity;
- Recombinant manufacturing was successful for the original ALT protein;
- Manual SPPS allowed higher degree of control over the process, yielding material of highest purity



DS: Lessons learned in Analytical Development

Analytical

- Investing in analytical method development early on in the program avoids pitfalls
  - High purity by generic HPLC method translated into lower purity by peptide specific, extended UPLC/ MS based analysis
- Impurity profiles heavily depend on manufacturing route

CRO#1 Purity claim (HPLC-UV) **98%**

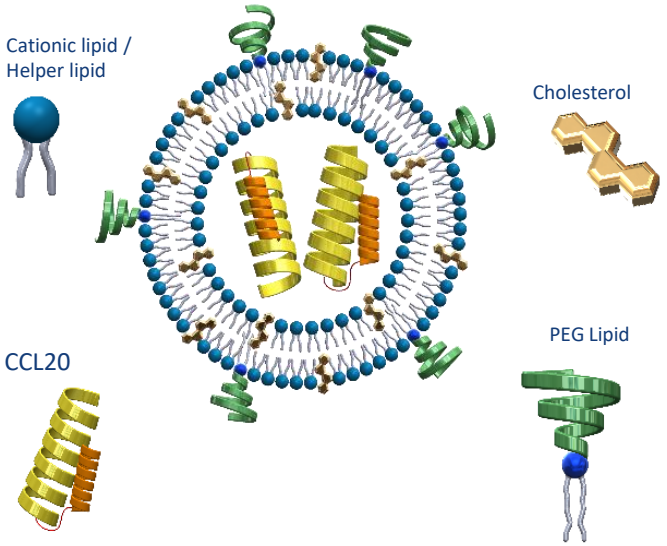
CRO#3 Re-analysis (UPLC-UV) **26%**

CRO#2 Re-analysis (LC-MS) **94%**

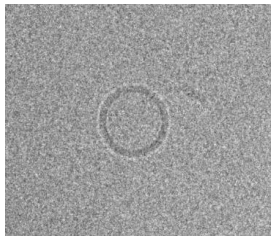
CRO#4 Re-analysis (UPLC-UV/MS) **21%**

CONCARLO THERAPEUTICS

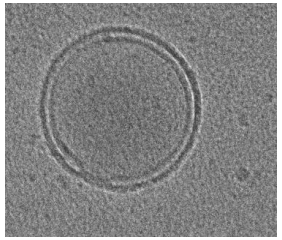
DP: Characterization of Fusogenic Liposomes for Intercellular Peptide Delivery



- Particle Size and PDI
- Lipid Molar Ratios
- Peptide : Lipid Ratio
- Surface properties: Zeta Potential
- Particle Morphology
- Stability
- In vitro potency and cytotoxicity (multiple formulation candidates)



Cryo-TEM: Empty Liposomes (60 – 90 nm)

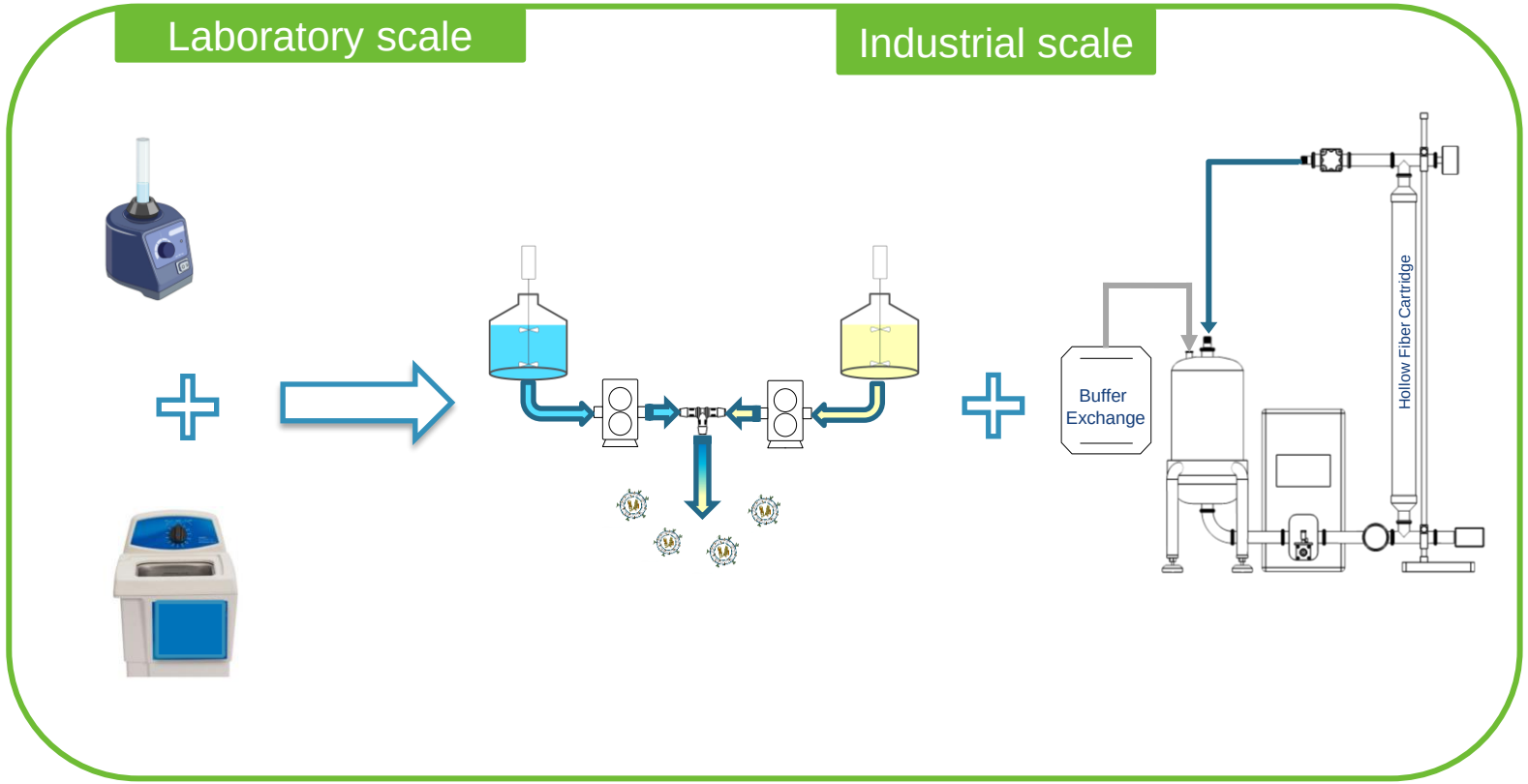


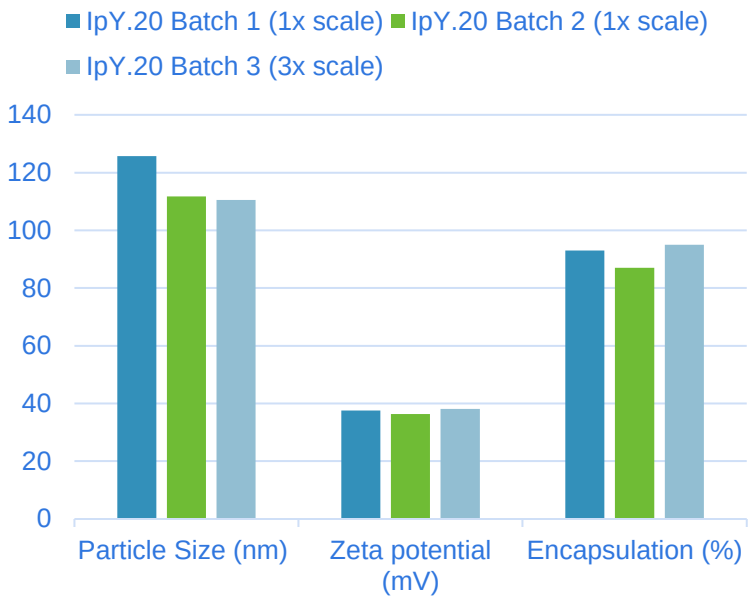
Cryo-TEM: Peptide Loaded Liposomes (100 – 130 nm)

CONCARLO THERAPEUTICS

DP: Transitioning from Lab Scale to Industry Standard Process

Particle Formation → **Purification** → **Aseptic Fill/Finish** → **Storage and Stability**





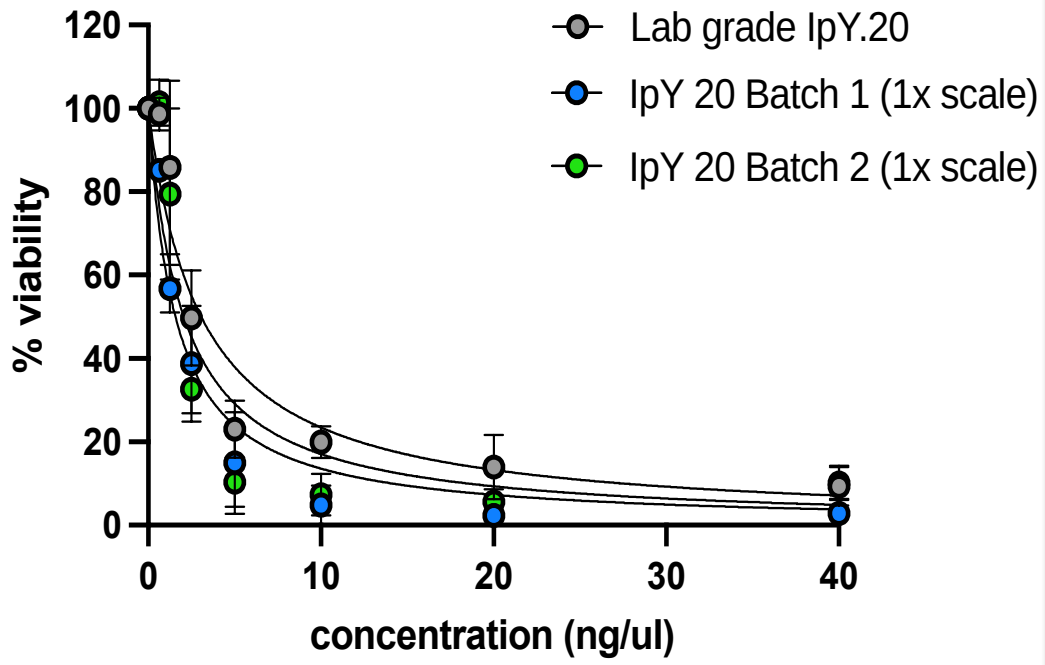
CONCARLO THERAPEUTICS

DP: Overcoming Challenges in Liposomal Manufacturing

Factors to keep in mind

- Exact mixing conditions differ between Laboratory scale and Production Scale
- Low lipid/peptide concentrations required for microfluidic mixing lead to large in-process volumes
- Longer processing times between steps can impact intermediate product stability
- Maintaining peptide conformation during LNP formation critical for peptide performance
- Minimizing effect of ethanol removal on particle size and stability during diafiltration
- Combined impacts of shear and product concentration during final ultrafiltration

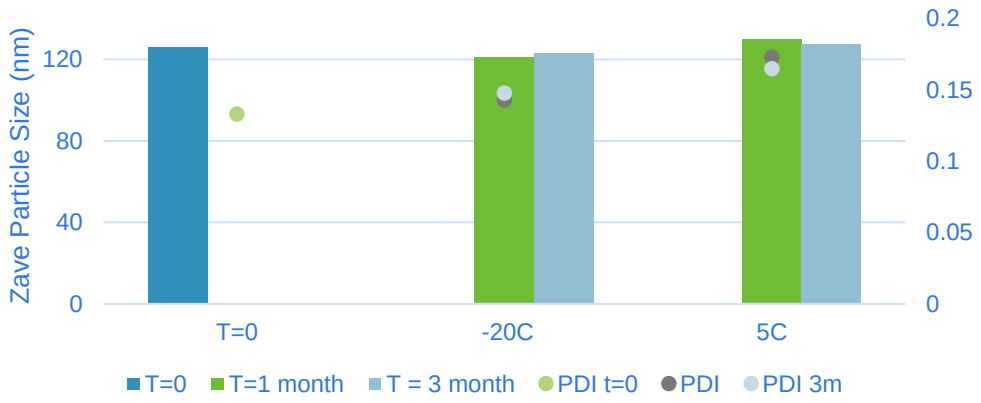
In Vitro Screening in ER+ breast cancer cell line

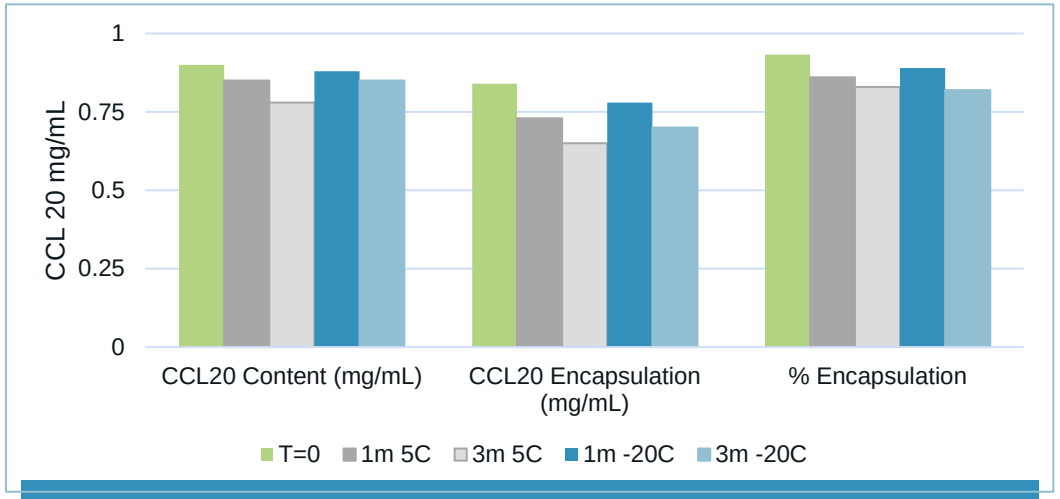


CONCARLO THERAPEUTICS

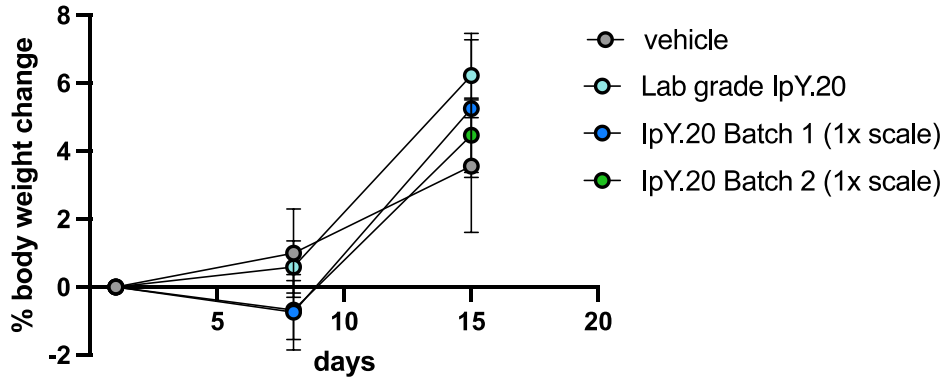
DP: IpY.20 stable at – 20ºC, simplifying cold chain requirements

On-Going Stability Studies: Product stable for 1m at 5ºC and -20ºC, and 3m at -20ºC

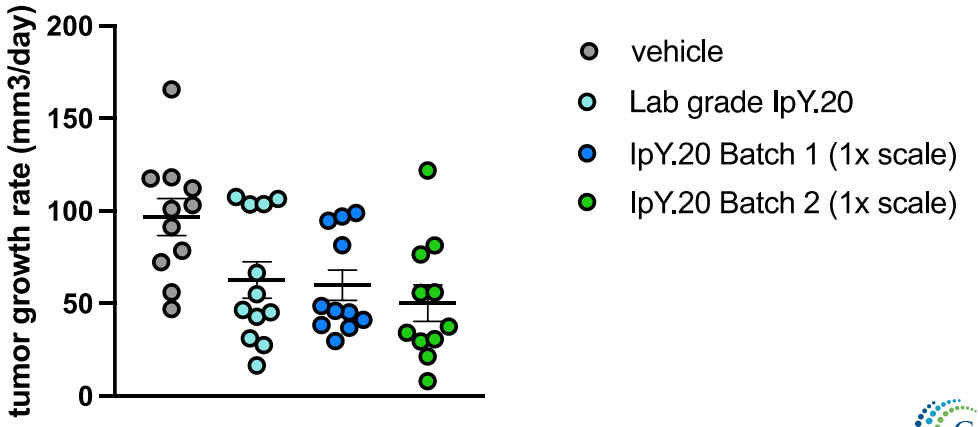




New IpY20 as tolerable as prior lab grade material

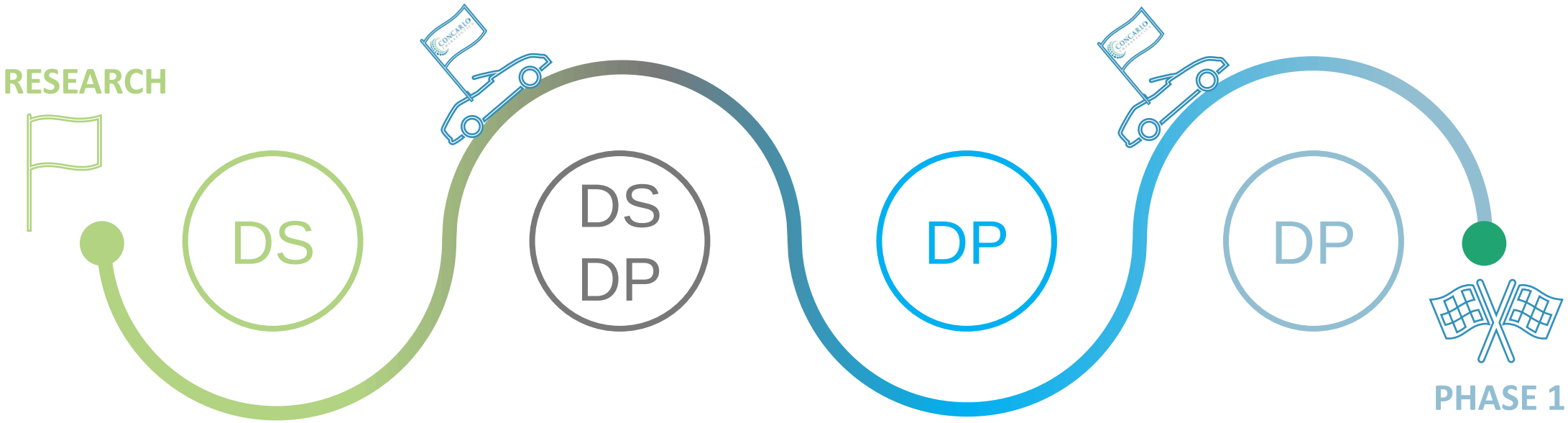


Scaled up IpY20 reduces tumor growth



Source: Concarlo, unpublished

IpY.20 progressing towards an IND-enabling program



PROPERTIES

Improved Solubility

Improved Purity

MANUFACTURING

DS: Multiple manufacturing Approaches Evaluated

DS: Manufacturing scaled by 20x

DP: Developed scalable process

DP: Product comparable to lab scale

EVALUATION

In Progress

In vitro screening

Preclinical PK / PD

Preclinical Efficacy and Toxicology

CHARACTERIZATION

Methods Developed

Size / Charge

Drug to Lipid Ratio

Encapsulation Efficiency

Morphology by Cryo-TEM

CONCARLO THERAPEUTICS

Acknowledgements

Concarlo Team:

- **Stacy W. Blain**
- Krishna Allamneni
- Dominique Bridon
- Grace Chen
- Anusha Aditya
- Lena Stafford
- Hassan Rashidzadeh

Contact Us!

We're hiring!

jenimperial@concarlo.com

CONCARLO THERAPEUTICS