3/8/21

Since we met:

1. MET inhibitors arrived, 10mM Crizotinib and Cabozantinib in DMSO
2. Finished barcoding library (actually right this time!!!! 🙌)
3. Cloned MET mutants from pUC19 in to pMSCV for mammalian cell retrovirus infection
4. Prepping cells/ inhibitor experiments and stable cell generation

To do:

1. Submit DNA for PacBio sequencing (by Friday hopefully)
    - Prepping sequences with SMRTbell adapters
    - Get sequencing data, analyze, do DMS!!!
2. Determine BaF3 IC50 values with Crizo and Cabo
3. Generate retrovirus and transduce BaF3 cells with MET kinase domain mutants

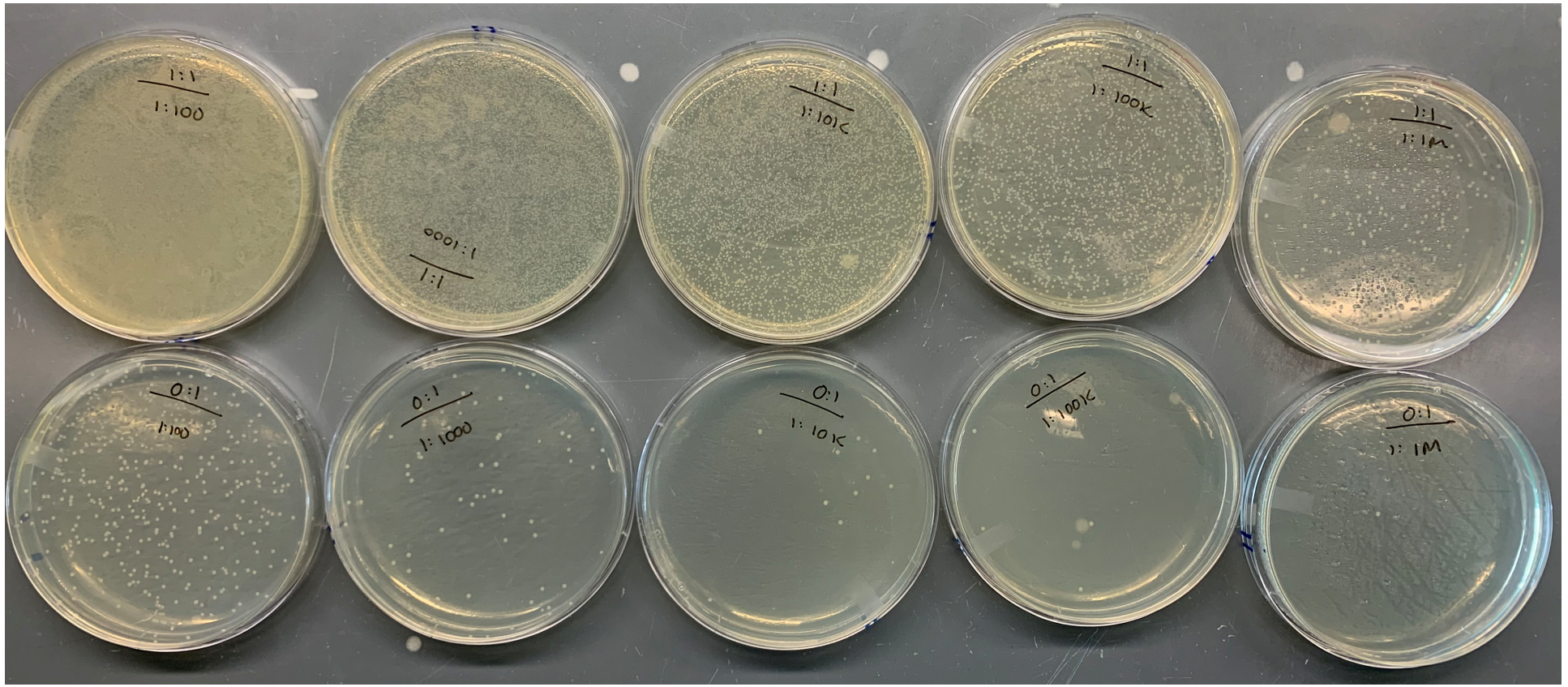
Barcoding the MET variant library

Brainstorming:
- say you have 4.0E+7 colonies in 1ml of cells
- that means you have 40,000 colonies in 1ul
- let's say you make a dilution such that you have 574 cells/ul in 5ml (adding 72ul of cell suspension into 5mL of LB)
- then to have 5740 cells in 25ml, you'd need 10ul of the 574 cells/ul dilution

Plate above dilutions on CARB+LB plates
1:100, 1:1k, 1:10k, 1:100k, 1:1M

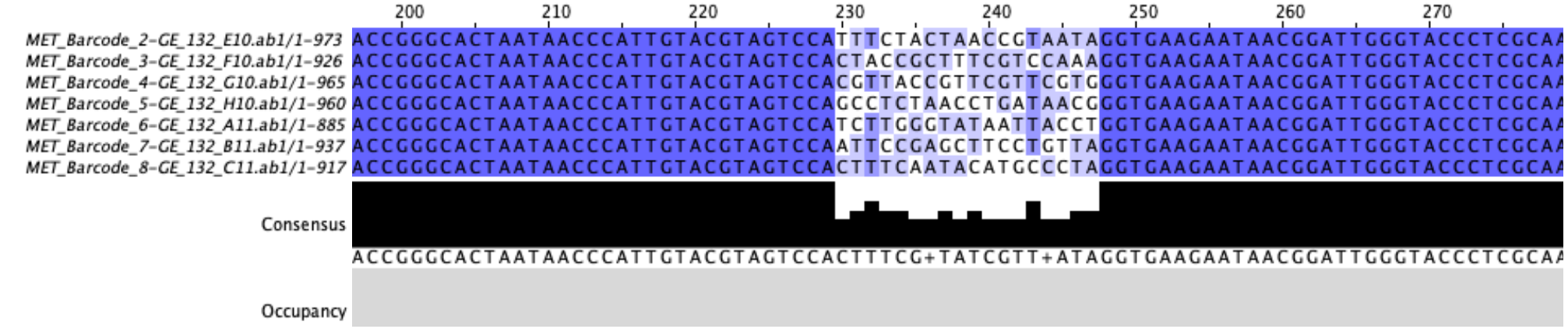
Library:Barcode Ratio	Estimate # Barcodes	Barcode Dilution (574 cells/ul stock)
1:1	5740	10 ul
1:2	11480	20 ul
1:5	28700	50 ul
1:10	57400	100 ul
1:20	114800	200 ul
1:40	229600	400 ul

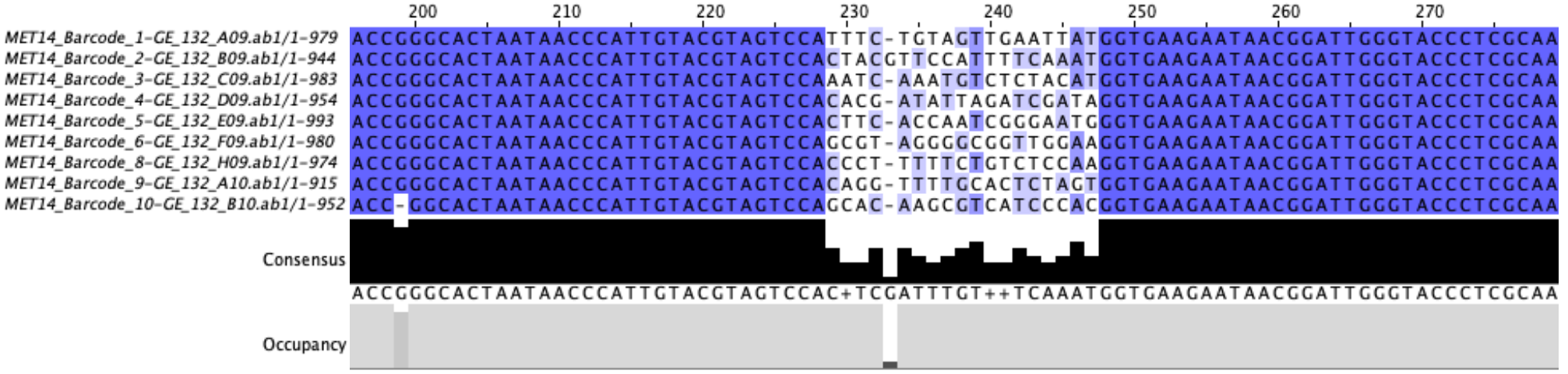
Barcoded library



- 1:1M colony count ~220 colonies
- TE = Colonies/µg/Dilution = 2.2e+9 cfu/ug = 220e+7 cfu
- This means the dilutions 1:1 (really 1:10), 1:2 (really 1:20), and 1:5 (really 1:50), 1:10 (really 1:100)

Last barcoded library sanger sequenced





Crizotinib and Cabozantinib are MET inhibitors

Journal of Medicinal Chemistry

ARTICLE

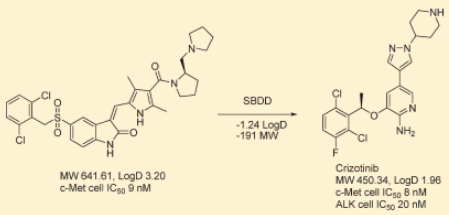
pubs.acs.org/jmc

Structure Based Drug Design of Crizotinib (PF-02341066), a Potent and Selective Dual Inhibitor of Mesenchymal−Epithelial Transition Factor (c-MET) Kinase and Anaplastic Lymphoma Kinase (ALK)†

J. Jean Cui,* Michelle Tran-Dubé, Hong Shen, Mitchell Nambu, Pei-Pei Kung, Mason Pairish, Lei Jia, Jerry Meng, Lee Funk, Iriny Botrous, Michele McTigue, Neil Grodsky, Kevin Ryan, Ellen Padrique, Gordon Alton, Sergei Timofeevski, Shinji Yamazaki, Qiuhua Li, Helen Zou, James Christensen, Barbara Mroczkowski, Steve Bender, Robert S. Kania, and Martin P. Edwards

La Jolla Laboratories, Pfizer Worldwide Research and Development, 10770 Science Center Drive, San Diego, California 92121, United States

ABSTRACT: Because of the critical roles of aberrant signaling in cancer, both c-MET and ALK receptor tyrosine kinases are attractive oncology targets for therapeutic intervention. The cocrystal structure of **3** (PHA-665752), bound to c-MET kinase domain, revealed a novel ATP site environment, which served as the target to guide parallel, multiattribute drug design. A novel 2-amino-5-aryl-3-benzyloxypyridine series was created to more effectively make the key interactions achieved with **3**. In the novel series, the 2-aminopyridine core allowed a 3-benzyloxy group to reach into the same pocket as the 2,6-dichlorophenyl group of **3** via a more direct vector and thus with a better ligand efficiency (LE). Further optimization of the lead series generated the clinical candidate crizotinib (PF-02341066), which demonstrated potent in vitro and in vivo c-MET kinase and ALK inhibition, effective tumor growth inhibition, and good pharmaceutical properties.



Molecular Cancer Therapeutics

Preclinical Development

Cabozantinib (XL184), a Novel MET and VEGFR2 Inhibitor, Simultaneously Suppresses Metastasis, Angiogenesis, and Tumor Growth

F. Michael Yakes, Jason Chen, Jenny Tan, Kyoko Yamaguchi, Yongchang Shi, Peiwen Yu, Fawn Qian, Felix Chu, Frauke Bentzien, Belinda Cancilla, Jessica Orf, Andrew You, A. Douglas Laird, Stefan Engst, Lillian Lee, Justin Lesch, Yu-Chien Chou, and Alison H. Joly

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

Co-occurring Alterations in the RAS–MAPK Pathway Limit Response to MET Inhibitor Treatment in MET Exon 14 Skipping Mutation-Positive Lung Cancer

Julia K. Rotow[1,2], Philippe Gui[1,2], Wei Wu[1,2], Victoria M. Raymond[3], Richard B. Lanman[3], Frederic J. Kaye[4], Nir Peled[5], Ferran Fece de la Cruz[6], Brandon Nadres[6], Ryan B. Corcoran[6], Iwei Yeh[2,7], Boris C. Bastian[2,7], Petr Starostik[8], Kimberly Newsom[8], Victor R. Olivas[1], Alexander M. Wolff[9], James S. Fraser[9], Eric A. Collisson[1,2], Caroline E. McCoach[1,2], D. Ross Camidge[10], Jose Pacheco[10], Lyudmila Bazhenova[11], Tianhong Li[12], Trever G. Bivona[1,2], and Collin M. Blakely[1,2]

TPR-MET IC50 experiments

Bivona lab IC50 assay using BaF3 and METΔEX14 mutation:

- Wash out IL3 with PBS and centrifugation.
- 96 well plate; seed 5000 cells/well
- Following day add RTKi from 0 - 10μM
- Collect at 72hrs and do the Cell Titer Glo assay
- Looked at full-length MET, supplemented with HGF and KRAS overexpression

What I propose:

- MET's lit IC50 values: criztoinbib 8nM, cabozantinib 0.035nM
- Test three BaF3 cell lines: MSCV empty, TPR-MET, TPR-MET+Ex14
- Test two media conditions: +IL3 (control), -IL3 (experimental)
- Test three inhibitor concentrations: 0.5nM, 5nM, and 50nM
- 72hr experiment: day1 plate conditions, day3 perform a cell titer glo assay
- Perform experiment in triplicate (technical replicates from pooled wells)

TPR-MET IC50 plate setup

Seeding Plate – 24 well

	+IL3			-IL3		
empty	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET+Ex14	0.5nM	5nM	50nM	0.5nM	5nM	50nM

1mL volumes
Inhibitor diluted in media

TPR-MET IC50 96-well glo assay setup

	+IL3			-IL3		
empty (1)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
empty (2)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
empty (3)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET (1)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET (2)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET (3)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET+Ex14 (1)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET+Ex14 (2)	0.5nM	5nM	50nM	0.5nM	5nM	50nM
TPR-MET+Ex14 (3)	0.5nM	5nM	50nM	0.5nM	5nM	50nM

- 50ul cell volume
- 50ul reagent volume