First report for the members of the Force Foundation Board

Systematic identification of small therapeutic peptides targeting vulnerabilities in fusion-driven pediatric, adolescent, and young adult sarcoma

Orthopedic Tumor Research, University Hospital Balgrist, University Zurich

Principal investigator: Prof. Dr. Didier Surdez,

PhD Student: Michelle Hofer, started March 2024

In the battle against cancer, various methods are used to identify strong and specific cancer dependencies, such as genome-wide pooled CRISPR screening (Dharia et al., 2021; Sun et al., 2023), RNA interference (McDonald et al., 2017), and drug response profiling (Corsello et al., 2020; Garnett et al., 2012). Despite intensive efforts to incorporate all generated data (Pacini et al., 2024), only very few small molecules targeting top dependency hits are available, especially in fusion-driven pediatric, adolescent, and young adult cancers. This is mainly attributed to the low efficiency and affinity of small molecules to bind to protein-protein interaction interfaces and the subsequent lack of inhibition (Lee et al., 2019; Lu et al., 2020). However, it has already been shown in the late 1990s that fragmented or truncated proteins and peptides can interfere with the native protein's interaction partners (Akada et al., 1997; Barnard et al., 1998). Therefore, interest in peptides as drugs and in the development of small molecules for inhibitors of protein-protein interaction has grown over the past years (Muttenthaler et al., 2021; Nicze et al., 2024; Sorolla et al., 2020).

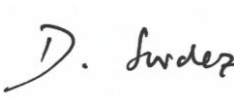
In this project, we are investigating inhibitory peptides of previously identified cancer dependencies (DepMap data) in Ewing sarcoma and fusion-positive alveolar Rhabdomyosarcoma. To systematically screen the top cancer dependency hits, the inhibitory peptides are designed as overlapping fragments of 40 amino acids, covering the full-length native protein. We will use next-generation sequencing to compare peptide presence at the culture end-point (day 14) with initial peptide coverage after transduction and use the fold-change to map bioactive domains. Peptides that cause a decrease in cell fitness can be exploited for efficient protein-protein interaction inhibition (PepTile system, see Ford et al., 2021). In addition to the linear peptide, we further designed a PROTAC-similar system, with the intent of increased degradation of the peptide binding proteins. Based on previously developed bioPROTAC structures, we use the peptide-protein interaction for targeted protein degradation instead of small molecules (Békés et al., 2022; Kim et al., 2024; Wang et al., 2023).

Current state of the project:

A PhD position for this project was advertised at the UZH PhD program (Summer 2023), but regrettably, no suitable candidate was found through this call. Fortunately, Michelle Hofer completed her Master's degree in our laboratory in the meantime (January 2023 - January 2024). She proved to be an excellent student and agreed to continue working in the lab on this PhD project. Ms. Hofer passed the entrance exam for the UZH Doctoral School of Oncology in December 2023 and defended her Master's thesis on February 29, 2024 (with a grade of 6.0). The following day, March 1St, Ms. Hofer officially started this project in the laboratory. Within two months, she has designed and cloned six constructs necessary for this project. In the next few weeks, she will perform pilot experiments for the different screening approaches. In particular, she will validate if the correct open reading frame is expressed and titrate the expression levels in our cell models using a few selected peptides. Next, the oligo pool library will be ordered and cloned into our backbones for systematic screening. **Perspective:** Following the analysis of the peptide screens using different approaches in Ewing sarcoma and rhabdomyosarcoma, we intend to investigate a subset of inhibitory peptides on the stability of the EWS-FLI1 fusion protein in the A673 cell line (Halo-tagged EWS-FLI1 model).

Prof. Didier Surdez,

Zurich, April 30th 2024



Bibliography

Akada, R., Yamamoto, J., & Yamashita, I. (1997). Screening and identification of yeast sequences that cause growth inhibition when overexpressed. Mol. Gen. Genet., 254, 267–274.

Barnard, D., Sun, H., Baker, L., & Marshall, M. S. (1998). In Vitro Inhibition of Ras-Raf Association by Short Peptides. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 247, 176–180.

Békés, M., Langley, D. R., & Crews, C. M. (2022). PROTAC targeted protein degraders: the past is prologue. Nature Reviews Drug Discovery, 21, 181–200. https://doi.org/10.1038/s41573-021-00371-6

Corsello, S. M., Nagari, R. T., Spangler, R. D., Rossen, J., Kocak, M., Bryan, J. G., Humeidi, R., Peck, D., Wu, X., Tang, A. A., Wang, V. M., Bender, S. A., Lemire, E., Narayan, R., Montgomery, P., Ben-David, U., Garvie, C. W., Chen, Y., Rees, M. G., … Golub, T. R. (2020). Discovering the anticancer potential of non-oncology drugs by systematic viability profiling. Nature Cancer, 1, 235–248. https://doi.org/10.1038/s43018-019-0018-6

Dharia, N. V., Kugener, G., Guenther, L. M., Malone, C. F., Durbin, A. D., Hong, A. L., Howard, T. P., Bandopadhayay, P., Wechsler, C. S., Fung, I., Warren, A. C., Dempster, J. M., Krill-Burger, J. M., Paolella, B. R., Moh, P., Jha, N., Tang, A., Montgomery, P., Boehm, J. S., … Stegmaier, K. (2021). A first-generation pediatric cancer dependency map. Nature Genetics, 53(4), 529–538. https://doi.org/10.1038/s41588-021-00819-w

Ford, K. M., Panwala, R., Chen, D. H., Portell, A., Palmer, N., & Mali, P. (2021). Peptide-tiling screens of cancer drivers reveal oncogenic protein domains and associated peptide inhibitors. Cell Systems, 12, 716–732. https://doi.org/10.1016/j.cels.2021.05.002

Garnett, M. J., Edelman, E. J., Heidorn, S. J., Greenman, C. D., Dastur, A., Lau, K. W., Greninger, P., Thompson, I. R., Luo, X., Soares, J., Liu, Q., Iorio, F., Surdez, D., Chen, L., Milano, R. J., Bignell, G. R., Tam, A. T., Davies, H., Stevenson, J. A., … Benes, C. H. (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature, 483, 570–575. https://doi.org/10.1038/nature11005

Kim, M. S., Bhargava, H. K., Shavey, G. E., Lim, W. A., El-Samad, H., & Ng, A. H. (2024). Degron-based bioPROTACs for controlling signaling in CAR T cells. BioRxiv Preprint. https://doi.org/10.1101/2024.02.16.580396

Lee, A. C. L., Harris, J. L., Khanna, K. K., & Hong, J. H. (2019). A Comprehensive Review on Current Advances in Peptide Drug Development and Design. International Journal of Molecular Sciences, 20(2383), 1–21. https://doi.org/10.3390/ijms20102383

Lu, H., Zhou, Q., He, J., Jiang, Z., Peng, C., Tong, R., & Shi, J. (2020). Recent advances in the development of protein–protein interactions modulators: mechanisms and clinical trials. Signal Transduction and Targeted Therapy, 5(213), 1–23. https://doi.org/10.1038/s41392-020-00315-3

McDonald, E. R., de Weck, A., Schlabach, M. R., Billy, E., Mavrakis, K. J., Hoffman, G. R., Belur, D., Castelletti, D., Frias, E., Gampa, K., Golji, J., Kao, I., Li, L., Megel, P., Perkins, T. A., Ramadan, N., Ruddy, D. A., Silver, S. J., Sovath, S., … Sellers, W. R. (2017). Project DRIVE: A Compendium of Cancer Dependencies and Synthetic Lethal Relationships Uncovered by Large-Scale, Deep RNAi Screening. Cell, 170, 577–592. https://doi.org/10.1016/j.cell.2017.07.005

Muttenthaler, M., King, G. F., Adams, D. J., & Alewood, P. F. (2021). Trends in peptide drug discovery. Nature Reviews Drug Discovery, 20, 309–325. https://doi.org/10.1038/s41573-020-00135-8

Nicze, M., Borówka, M., Dec, A., Niemiec, A., Bułdak, Ł., & Okopień, B. (2024). The Current and Promising Oral Delivery Methods for Protein- and Peptide-Based Drugs. International Journal of Molecular Sciences, 25(815), 1–29. https://doi.org/10.3390/ijms25020815

Pacini, C., Duncan, E., Gonçalves, E., Gilbert, J., Bhosle, S., Horswell, S., Karakoc, E., Lightfoot, H., Curry, E., Muyas, F., Bouaboula, M., Pedamallu, C. S., Cortes-Ciriano, I., Behan, F. M., Zalmas, L. P., Barthorpe, A., Francies, H., Rowley, S., Pollard, J., … Garnett, M. J. (2024). A comprehensive clinically informed map of dependencies in cancer cells and framework for target prioritization. Cancer Cell, 42, 301–316. https://doi.org/10.1016/j.ccell.2023.12.016

Sorolla, A., Wang, E., Golden, E., Duffy, C., Henriques, S. T., Redfern, A. D., & Blancafort, P. (2020). Precision medicine by designer interference peptides: applications in oncology and molecular therapeutics. Oncogene, 39, 1167–1184. https://doi.org/10.1038/s41388-019-1056-3

Sun, C. X., Daniel, P., Bradshaw, G., Shi, H., Loi, M., Chew, N., Parackal, S., Tsui, V., Liang, Y., Koptyra, M., Adjumain, S., Sun, C., Chong, W. C., Fernando, D., Drinkwater, C., Tourchi, M., Habarakada, D., Sooraj, D., Carvalho, D., … Firestein, R. (2023). Generation and multi-dimensional profiling of a childhood cancer cell line atlas defines new therapeutic opportunities. Cancer Cell, 41, 660–677. https://doi.org/10.1016/j.ccell.2023.03.007

Wang, H., Zhou, R., Xu, F., Yang, K., Zheng, L., Zhao, P., Shi, G., Dai, L., Xu, C., Yu, L., Li, Z., Wang, J., & Wang, J. (2023). Beyond canonical PROTAC: biological targeted protein degradation (bioTPD). Biomaterials Research, 27(72), 1–28. https://doi.org/10.1186/s40824-023-00385-8