**PROJECT**

| | |
|---|---|
| **Supervisor:** | Davide Gabellini |
| Title: | Understanding and targeting the DUX4-r oncogenic pathway |
| *Curriculum*: | Cell and Molecular Biology |
| Link to the personal page of the University or relevant hospital site website: | https://research.hsr.it/en/divisions/genetics-and-cell-biology/gene-expression-regulation.html |

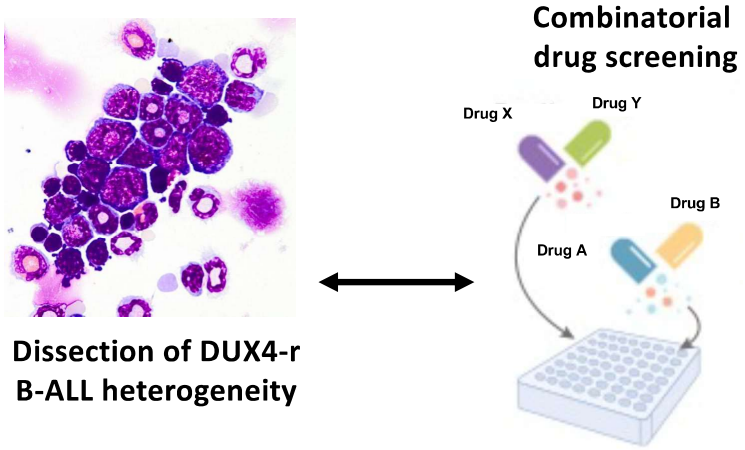
**Description of the Project (max 3,000 characters including spaces)**

| |
|---|
| |

| | |
|---|---|
| Title: | Understanding and targeting the DUX4-r oncogenic pathway |
| *Curriculum*: | Cell and Molecular Biology |
| Link to the personal page of the University or relevant hospital site website: | https://research.hsr.it/en/divisions/genetics-and-cell-biology/gene-expression-regulation.html |

**Description of the Project (max 3,000 characters including spaces)**

| |
|---|
| |



**Background/gap of knowledge**

B-cell acute lymphoblastic leukemia (B-ALL) is the most common pediatric cancer and the major cause of cancer-related death among children and young adults. Despite substantial progress, patients not responding to current therapeutics have a poor outcome (1).

Translocations giving rise to rearranged versions of the double homeobox 4 transcription factor (DUX4-r) are responsible for a significant fraction of B-ALL cases (2, 3).

We found that DUX4-r blocks B-cell differentiation and directly activates the expression of oncogenes and other factors driving cell adhesion, migration, tumor spheroid formation, survival, proliferation and other cancer pathways (4).

**Rationale and hypothesis**

The DUX4-r subtype is underdiagnosed and poorly characterized. Consequently, no targeted therapy currently exists and relapse cases face a dismal prognosis.

Our recent characterization of the oncogenic DUX4-r gene signature and the cellular consequences of DUX4-r overexpression in leukemia (4) put us in a privileged position to further dissect the DUX4-r oncogenic mechanism and identify possible treatments. Based on our published and preliminary results, we hypothesize that the direct DUX4-r targets are a rich source of novel diagnostic, prognostic and therapeutic targets.

**Objectives and specific aims**

While we showed that the ability of DUX4-r to activate its direct targets is required for leukemogenesis (4), the contribution of these targets to DUX4-r oncogenic activity is currently unknown.

**1. Characterization of factors affecting DUX4-r B-ALL heterogeneity**

DUX4-r cases exhibit a high degree of lineage infidelity characterized by downregulation of B-cell markers, and multilineage priming toward nonhematopoietic, myeloid, and T-cell lineages, which is associated to lower treatment response. We and others have identified several possible factors that could mediate this heterogeneity.

To evaluate the above candidates, their level will be modulated in cellular and animal models of DUX4-r leukemia, which will be characterized at phenotypic and molecular levels.

**2. Identification of drug combinations synergistically killing DUX4-r leukemia cells**

Combining multiple drugs can allow to identify synergistic interactions and reach optimal efficacy with fewer side effects. Intriguingly, FDA-approved drugs are available for many direct DUX4-r targets.

Here, selected drugs drug will be tested either individually or in combination in cellular models. Next, the best combinations will be tested in animal models of DUX4-r leukemia. For the best performing treatments, we will identify the affected pathways and the effect on the DUX4-r oncogenic signature.

**Expected outcomes**

The project will clarify the disease mechanism, and could help identifying prognostic factors, and refine risk stratification. Moreover, we could identify clinically relevant targeting modalities displaying efficacy toward DUX4-r leukemia with minimal side effects, which will open the potential for de-escalating current chemotherapeutic regimens in DUX4-r B-ALL.

**Skills that the student should acquire** (max. 600 characters including spaces):

The project entails a broad range of approaches, including lentiviral targeting, drug screening, next generation sequencing, cell biology, flow cytometry, immunoassays and animal models of disease. The student will learn how to critically evaluate the literature, design experiments, discuss the obtained results and assess their biological relevance. Training will also concern how to present scientific results at scientific meetings and write manuscripts or funding applications.

**References** (max. 15)

1. Pagliaro,L., Chen,S.J., Herranz,D., Mecucci,C., Harrison,C.J., Mullighan,C.G., Zhang,M., Chen,Z., Boissel,N., Winter,S.S., et al. Acute lymphoblastic leukaemia. (2024) Nat Rev Dis Primers, 10.

2. Yasuda,T., Tsuzuki,S., Kawazu,M., Hayakawa,F., Kojima,S., Ueno,T., Imoto,N., Kohsaka,S., Kunita,A., Doi,K., et al. Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. (2016) Nat Genetics, 48, 569–574.

3. Carlet,M., Völse,K., Vergalli,J., Becker,M., Herold,T., Arner,A., Senft,D., Jurinovic,V., Liu,W.H., Gao,Y., et al. In vivo inducible reverse genetics in patients' tumors to identify individual therapeutic targets. (2021) Nat Commun, 12.

4. Campolungo,D., Salomé,M., Biferali,B., Tascini,A.S. and Gabellini,D. DUX4-r exerts a neomorphic activity that depends on GTF2I in acute lymphoblastic leukemia. (2023) Sci Adv, 9.