Collaborative Transversal Project – CIBERONC Network, Liquid Biopsy WM

The Liquid Biopsy (LB) and Biomarker Working Module

The CIBERONC LB-WM is formed to **foster a transversal collaboration and LB research amongst the different programs of CIBERONC Network**, which is formed by researchers from six different programs, Gastrointestinal Tumors, Low Prevalence Tumors, Respiratory Tract Tumors, Breast Cancer, Haematological Tumors and Mechanisms of tumor progression.

The main objective of the CIBERONC LB-WM is to establish a platform for the standardization and promotion of the Liquid Biopsy within the CIBERONC network.

Specific aims

1. To serve as a hub to provide information about the laboratories and researchers participating in the network of the Liquid Biopsy field.
2. Provide standardized liquid biopsy protocols, and additional technological tools
3. Provide updates with the most recent LB research findings and collaboration opportunities.
4. Interface to engage human and technological resources and promote interaction between CIBERONC groups.
5. Promote the understanding of the liquid biopsy applications for clinicians and patients

1. Project

Deciphering the genetic profiles of responders and resistant tumors to immune checkpoint inhibitors using tumor and plasma cfDNA exome sequencing

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Leading CIBERONC Working Modules: Liquid Biopsy

Collaborating CIBERONC Working Modules: Immuno-Oncology, Cancer Bioinformatics & Omics

Funding: CIBERONC WM (€ 50.000) and Swiss Bridge Award (€ 200.000)

Institutional Ethics Committee: Vall d'Hebron University Hospital

2. Scientific Context

Tumor Mutation Burden

There are inconsistent results of Tumor Mutation Burden (TMB) as a potential biomarker of response to immunotherapy. The initial assumption was that TMB could be a surrogate measure of neoantigens and potential immunogenicity of tumors and their visibility by the immune system. Although some TMB analyses of non-small cell lung cancer (NSCLC), melanoma, urothelial cancers, and solid tumors showed to be a potential predictor biomarker for response to immune checkpoint inhibitor (ICI) and possibly advanced overall survival (OS), the TMB remains a still relatively new biomarker with several aspects that are still poorly understood.

Beyond TMB

Beyond TMB, other genomic patterns such as type of mutations, tumor mutation signatures, number of predicted neoantigens, and enrichment for specific genes in the responders or refractory groups have been less explored in the context of ICI. While gene panel platforms as the MSK-IMPACT panel (468 genes, 1.2 Mb), the Foundation Medicine Panel (315 genes, 1.2 Mb), the Oncomine Tumor Mutation Load Assay (Life Technologies; 409 genes, 1.7 Mb) and the TruSight

Tumor 170 (Illumina; 170 genes, 0.5 Mb) are easier to analyze, the optimal platform for broader analyses is whole-exome sequencing (WES) that comprises 22,000 genes and up to 50 Mb.

We here propose an Innovative Sequencing Strategy: Whole exome-sequencing of germline DNA (gDNA), tumor DNA (tDNA – when available) and plasma cell-free DNA (cfDNA) will be performed to the identification of cancer- exclusive (somatic) mutations, according to protocols recently reported from groups of CIBERONC. Overall, the concordance rates of tumor and cfDNA exome sequencing in metastatic cancer patients range between 75% to 90% (Toledo RA *et al*. Clin Cancer Res. 2018; 24:3550-3559). Interestingly, cfDNA exome sequencing was capable of detecting somatic mutations nondetectable by standard tumor exome sequencing, most likely due to genetic heterogeneity of patients' different tumor lesions.

3. Project Aims

1) We aim to use the expertise of the CIBERONC network on plasma cfDNA sequencing and capacity to enroll patients in translational studies to characterize the tumor genomic profiles of responders and not responders to immunotherapy.

a. To this, we will sequence all the 20,000 genes of the human genome of cfDNA and gDNA (and tDNA when available) of patients treated with immunotherapy, and relate the results to clinical response.

2) We aim to use the exome sequencing data to unbiasedly investigate the influence of tumor mutation load, different types of mutation, tumor mutation signatures, and whether specific genes are enriched in the cohort of responders or refractory patients.

4. Preliminary results:

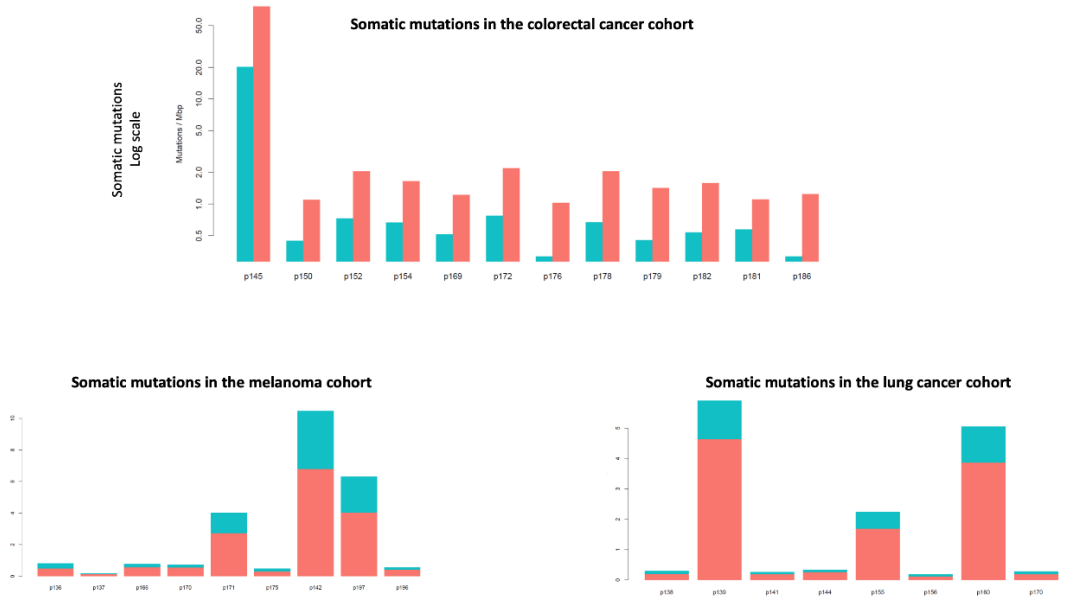

Figure 1: Whole-exome sequencing of basal plasma cfDNA and gDNA of 50 patients with metastatic cancer) treated with immunotherapy have been performed. Preliminary bioinformatics analysis has been completed and tumor mutations and CNV have been identified.

5. Goal

After finishing the proof-of-concept of the project with these 50 patients (most of the sequencing were funded by the Swiss Bridge Foundation grant), the recruitment of the project is now expanded to the whole CIBERONC network. As the objective to the projects involving CIBERONC working modules, the project is highly transversal and accepts the inclusion of patients with any type of cancer, following the inclusion/exclusion criteria below:

6. Inclusion criteria

Patient needs to meet all the inclusion criteria:

1) Patient with any tumor type with metastatic disease, treated with anti-PD1 or anti-PDL1 in monotherapy or in combination (needs to have an anti-PD1 and/or anti-PDL1 backbone, any line of treatment is accepted).

2) Fulfils at least one of the following criteria.

a. Clinical benefit with immunotherapy (PR, CR, SD >6months).

b. Known to be MSI high by PCR or IHC (independent of response to ICI).

c. Known to be tumor mutation burden high (independent of response to ICI).

d. Known to harbor a pathogenic mutation in POLE/D (independent of response to ICI).

e. Known to harbor a (germline or somatic) pathogenic mutation in a DNA repair gene (independent of response to ICI).

3) To have the following samples available:

a. Normal DNA or sample for extraction of normal DNA (saliva, fresh/frozen blood, fresh/frozen PBMCs – collected at any time).

b. Baseline plasma sample (from immediately prior to ICI)

4) Patient should have signed an Institutional informed consent IRB-approved that allows sample collection and sharing with other institutions.

5) Collaborators must fill in a spreadsheet provided by the investigators with clinical and therapeutic data of the enrolled patients.

Optional: Tumor sample (frozen/FFPE)

7. Exclusion criteria:

Patient not evaluable for response after ICI (without a RECIST tumor assessment) ICI.

8. Samples requested

a) 5 mL of peripheral blood collected in a tube for cfDNA preservation (Cell-Free DNA BCT, or similar tube. Such tube can be shipped by the Investigator to the interested groups if needed). Peripheral blood collection using the provided tube; homogenization of blood with the buffer by inverting the tube 10X; ready to ship together with a copy of the informed consent. All procedures, including shipment, will be carried out at room temperature. The shipment has to be received by the lab at VHIO-Barcelona within maximum 72 hours after blood collection.

OR

b) Stored frozen plasma collected using cfDNA compatible protocols or 20-40ng of cfDNA can also be sent. In this case, please contact the researcher first for discussing more details.

9. Costs

Clinical/Translational groups with grants that cover sequencing costs will be asked to use such resources. The remaining CIBERONC grant will be preferentially used for sequencing of the samples of groups without sequencing grants. Shipping costs can be covered by the CIBERONC grant if needed.

10. Expected results

In line with our preliminary results, we expect to build a genomic dataset of metastatic cancer patients treated with ICI that can be used to discovery of potential biomarkers associated with response to these therapies.

11. Projected time frame

Deadline for sample / data acquisition:

3 - 6 months after the project is circulated to the CIBERONC Network members.

Deadline for data analysis:

3-6 months after sequencing is completed.

Expected manuscript submission:

Approx. 6 months after all sequencing and bioinformatics analyses are completed.

12. Targeted Journals (depending on outcome):

JAMA Oncology, Nature Medicine/Cancer/Communications, Cancer Discovery, CCR

13. Publication policy

Recipient scientist formally agrees with the provider(s) - at the time of the request or soon after the provider(s) have accepted the collaboration - the(ir) presence (if any) as co-authors in the publications originating from the collaboration.

Number of authors per Group is specified as follows:

1-10 patients with informative results and included in the paper = 1-2 authors

11-25 patients with informative results and included in the paper = 2-4 authors

>25 patients with informative results and included in the paper = >4 authors

Project leading will the first and last/corresponding authors. Order of authorships will vary according with the number of patients included and key participation on the project.

14. Conditions of data usage

The Recipient scientist will use the data for research purposes only.

The data will be used by the Recipient scientist solely in connection with the Research Project as outlined above.

The Recipient scientist shall use the data in compliance with all applicable laws and government regulations of the Recipient's country.

The Recipient Scientist shall not release the data to any person other than the personnel under the Recipient Scientist's direct supervision or directly involved in data analysis.

15. Conditions of biomaterial usage

Biomaterial received to participate in this proposal will be used only for achieving the aims specified above and related to the main goals of the study. Biomaterial not used will be return to the corresponding Group immediately after to the completion of the experiments.

16. Contact

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