Allegato / *Annex B*

PROGETTO DI RICERCA / RESEARCH PROJECT
(max 5 pagine / max 5 pages)

Cognome/Surname	CONTINO
Nome / Name	SILVIA
Titolo del progetto / Project title	The role of liquid biopsy to detect resistance mutations to TKI in metastatic Gastrointestinal Stromal Tumors (GISTs)
Corso di dottorato / PhD	ONCOLOGIA E CHIRURGIA SPERIMENTALI
Firma del candidato/ Applicant's signature	

1 - Abstract

Gastrointestinal Stromal Tumors (GISTs) represent an exact model of oncogene addicted cancer. The discovery of KIT and PDGFRA mutations represented a revolution in terms of disease treatment and in the knowledge of the molecular cancer biology. In the guidelines the mutational state is not included among the prognostic factors, however from the literature we know the crucial role they play. In this scenario, this study aims at evaluating whether there is the ability to identify the mutational state in ctDNA on metastatic patients, treated with TKI and KIT exon 11 mutation, compared to the presence of the mutation on tumor mass. Consequentially our second objective will be to study the mutational state, identified in the ctDNA, as a prognostic factor.

2 - Project

Background

Liquid biopsy is a powerful technique that can be applied to different stages of cancer screening and treatment, thanks to this analysis we can see on the blood of cancer patients the presence of circulating tumor DNA (ctDNA) [7]. Circulating tumor DNA (ctDNA) represents a portion of the cfDNA released by tumor cells through apoptosis, necrosis, or active release [8]. The concentration of ctDNA in plasma has been shown to correlate with tumor size and stage [11, 12]. We can see also different molecules, including circulating free DNA (cfDNA), circulating tumor cells (CTCs), circulating RNA (cRNA) and extracellular vesicles (EVs) [9]. Liquid biopsy offers repeatability due to its minimally invasive nature, which in turn leads to better acceptance by patients [7]. Liquid biopsy is successfully used in many tumors such as breast, lung, and colorectal cancer [10]. Recent studies have been investigating the role of liquid biopsy also in Gastrointestinal stromal tumors (GISTs). GISTs are rare, accounting for less than 1% of cancers [1], but they represent the most common mesenchymal tumor of the gastrointestinal tract [2]. Approximately 80% of GISTs harbor activating mutations in the KIT or PDGFRA genes that are responsible for the up regulation of crucial signaling

pathways [3]. The discovery of mutation on KIT and PDGFRA represented a revolution in the treatment of GIST, the tyrosine kinase inhibitors are successfully used. The first line of metastatic GIST is represented by a daily dose of imatinib (400 mg) [14]. However, responses to imatinib depend on the functional domain affected [14]. Patients with KIT exon 11 mutation have a partial response rate of 80%, whereas KIT exon 9 mutations are associated with a lower response rate of 40%. The patients with KIT exon 9 mutations benefit from the higher dose of imatinib (800 mg/die) [14,15]. The most frequent mutations in GISTs are located on KIT exon 11, these patients represent a heterogeneous subgroup in terms of biological and clinical behavior. From the literature it is known as the absence of exon 11 deletions or delins 557/558 are significant prognostic factors for longer PFS. Furthermore, the presence of exon 11 557/558 deletions/delins is characterized by an aggressive biology and allow for prediction at the baseline which GIST patients would develop resistance to first line imatinib treatment earlier [16]. This heterogeneity in mutational hotspots has slowed down liquid biopsy development and clinical use in GIST patients, unlike in other tumors such as lung cancer, in which it is being successfully implemented [5, 6].

Primary Objective

- The aim of this project is to study the prognostic and predictive role of liquid biopsy on metastatic GIST patients, with tumor harboring KIT exon 11 mutation, treated with TKI. The presence of the KIT 11 mutation on circulating tumor DNA (ctDNA) will be analyzed, and the results will be compared to the tumor tissue mutational analysis.

Secondary Objective

- To investigate the role of liquid biopsy in GIST to detect primary and secondary acquired mutations.

Patients and methods

Study Population

In our study we will include metastatic GIST patients treated with TKIs. The information, collected from these patients, will include gender, age, site of the origin of primary tumors, primitive tumor diameter and mitosis, KIT and PDGFRA pathogenic variant (PV) classification, data on active disease sites, best overall response (BOR) to imatinib, progression disease (PD), stable disease (SD), partial response (PR), complete response (CR) assessed according to response evaluation criteria in solid tumors (RECIST version 1.1.), progression-free survival (PFS) to imatinib treatment and overall survival (OS). The patients will be classified in 2 groups: i) KIT Exon 11 deletion or insertion/deletion in codons 557 and/or 558 (named “D-557/8”); ii) other mutations than D-557/8 (named “No-D-557/8”). Subsequently we are going to divide the group of other PVs than 557/558 deletions (NoD-557/8) into two further subgroups: (i) patients with KIT Exon 11 deletion or

delins in codons other than 557/558; (ii) patients with duplication, insertion or SNV of all codons. Signed written informed consent will be collected for every enrolled patient.

Methods

Plasma collection and ctDNA isolation

For each eligible patients, blood samples will be collected at baseline and after progression disease in four 3-mL EDTA-containing vacutainer tubes and processed within one hour for plasma collection. Whole blood samples will centrifugate at 4° C for 10 minutes at 1200g to eliminate cell debris and the obtained supernatant is then centrifuged again for 10 minutes at 3000g at 4° C. Plasma is then stored in cryostat tubes at -80°C until next use. Circulation DNA is isolated using QIAamp Circulating Nucleic Acid Kit (Qiagen) according to manufacturer's protocols for 2 mL plasma. DNA quantification is performed using both NanoDrop (Thermo Scientific) according to manufacturer's recommendation and Quibit 2.0 Fluorometer (Life Technologies) with the dsDNA HS (High Sensitivity) Assay Kit.

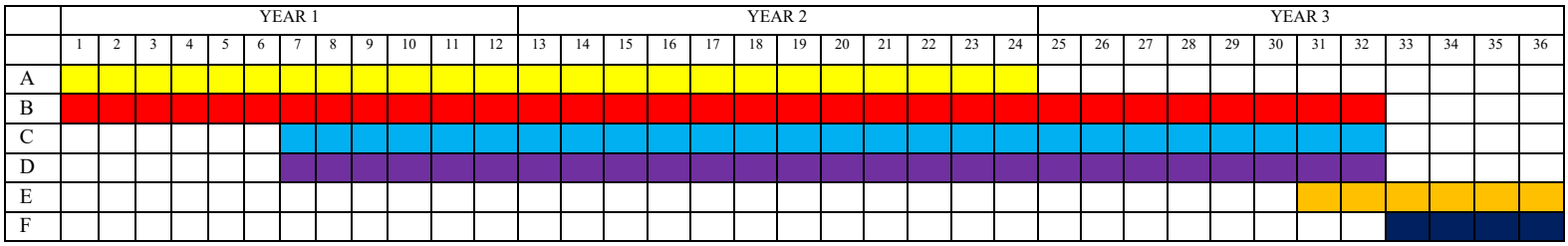
Mutational Analysis

To monitor the occurrence of resistance mutations (i.e., exon 17 D861V, exon 13 V654A, or novel secondary mutations, such as D820G and A829 in exons 17 and 18 of KIT, respectively) during the administration of Tyrosine-Kinase Inhibitors in GIST patients, serial plasma samples will be taken from patients in treatment with Imatinib during routine follow-up. In particular, patients carrying a c-KIT exon 11 pathological genetic variant will be selected and then subclassified on the basis of the harbored alteration considering the specific codon involved. The occurrence of secondary mutations will be evaluated through the QX200™ droplet digital PCR (ddPCR) (Bio-Rad) by using a validated or customized ddPCR MUT FAM+HEX Assay and the data will be analyzed and corrected by the QuantaSoft™ Software supplied with the instrument.

Statistical Analysis

PFS will be calculated from beginning the TKI treatment to death by any cause or disease progression or last follow-up (censored patients). OS will be calculated from beginning the imatinib to death by any cause or last follow-up (censored patients). The analysis of PFS and OS between groups will be compared using the Kaplan-Meier method and log-rank test. To identify independent prognostic factors for PFS and OS, univariate and multivariate Cox proportional hazard regression models will be built. The comparison between subgroups will be performed with Anova test, Fisher exact test, and Pearson's chi-square test.

Timeline



A. Patient's enrollment
B. Sample from blood collection
C. Genetic analysis
D. Scientific literature update
E. Statistical analysis
F. Article publication

Expected Results

Liquid biopsy is a powerful technique that can be applied to different stages of cancer screening and treatment, thanks to is minimally invasive nature is better acceptance by patients. The heterogeneity in mutational hotspots has slowed down liquid biopsy development and clinical use in GIST patients, this project could allow to demonstrate the possibility to introduction of this method in metastatic GIST patient. We expect to find a concordance between the mutational state in the ctDNA and in the tumor mass.

3 –References

1. Ray-Coquard, P. Cassier, H. El Sayadi, J.Y. Blay, Tumeurs gastro-intestinales (GIST), in: J.P. Droz, I. Ray-Coquard, J.L. Peix (Eds.), P630. Tumeurs Malignes Rares, Springer-Verlag, France, Paris, 2010, pp. 149–154.
2. B.P. Rubin, M.C. Heinrich, C.L. Corless, Gastrointestinal stromal tumour, Lancet 369 (2007) 1731–1741.
3. Liegl-Atzwanger, B.; Fletcher, J.A.; Fletcher, C.D. Gastrointestinal stromal tumors. *Virchows Arch. Int. J. Pathol.* **2010**, *456*, 111–127.
4. Iva Brcˇic´, Alexandra Argyropoulos and Bernadette Liegl-Atzwanger Update on Molecular Genetics of Gastrointestinal Stromal Tumors *Diagnostics* (Basel) 2021 Jan 28;11(2):194
5. Castellanos-Rizaldos E, Grimm DG, Tadigotla V*, et al*. Exosome-based detection of EGFR T790M in plasma from non-small cell lung cancer patients. *Clin Cancer Res* 2018; 24: 2944–2950.
6. Ravegnini G, Sammarini G, Serrano C, Nannini M, Pantaleo MA, Hrelia P, Angelini S. Clinical relevance of circulating molecules in cancer: focus on gastrointestinal stromal tumors. Ther Adv Med Oncol. 2019 Mar 1;11:1758835919831902. doi: 10.1177/1758835919831902. PMID: 30854029; PMCID: PMC6399766.

7. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, Pacey S, Baird R, Rosenfeld N. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223.
8. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA: apoptosis and active DNA release. Clin Chim Acta. 2001;313(1–2):139–42.
9. Siravegna G, Marsoni S, Siena S*, et al*. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol* 2017; 14: 531–48.
10. Hench IB, Hench J, Tolnay M. Liquid Biopsy in Clinical Management of Breast, Lung, and Colorectal Cancer. Front Med (Lausanne). 2018 Jan 30;5:9. doi: 10.3389/fmed.2018.00009. PMID: 29441349; PMCID: PMC5797586.
11. Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, Del Rio M, Molina F. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acids Res. 2010;38(18): 6159–75.
12. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, et al. Detection of circulating tumor DNA in early-and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra24.
13. Kelly CM, Gutierrez Sainz L, Chi P. The management of metastatic GIST: current standard and investigational therapeutics. J Hematol Oncol. 2021 Jan 5;14(1):2. doi: 10.1186/s13045-020-01026-6. PMID: 33402214; PMCID: PMC7786896.
14. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003; 21:4342–4349. [PubMed: 14645423]
15. Serrano, C.; George, S. Gastrointestinal Stromal Tumor: Challenges and Opportunities for a New Decade. *Clin. Cancer Res.* **2020**, *26*, 5078–5085.
16. Incorvaia L, Fanale D, Vincenzi B, De Luca I, Bartolotta TV, Cannella R, Pantuso G, Cabibi D, Russo A, Bazan V, Badalamenti G. Type and Gene Location of *KIT* Mutations Predict Progression-Free Survival to First-Line Imatinib in Gastrointestinal Stromal Tumors: A Look into the Exon. Cancers (Basel). 2021 Feb 27;13(5):993. doi: 10.3390/cancers13050993. PMID: 33673554; PMCID: PMC7956844.