Impact of a Molecular Sequencing Systematic at Diagnosis in Digestive Oncology: Experience of a French Center

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ABSTRACT

Introduction: Tumor-based molecular profiling has increased in the area of precision medicine. Their routine use is still limited by accessibility, cost and availability of tumor material.

Materials and Methods: We retrospectively analysed the treatment received and the survival data of patients with digestive cancer who received molecular high-throughput sequencing (NGS) analyses at diagnosis. The primary objective of this single-center study was to compare the overall survival of patients who were treated with molecularly matched therapy with patients who received standard therapy. Median overall survival was calculated from initial disease diagnosis to death.

Results: 528 patients were referred to the Digestive Oncology Department of the Timone Hospital in Marseille between January 2018 and November 2020 for management of digestive cancer and received high-throughput molecular sequencing. Among them, 461 patients had a digestive carcinoma (75 of them were excluded because of the presence of a GIST or a neuroendocrine tumor, a digestive localization of extra digestive cancer or the absence of follow-up in our center) and 275 had metastatic disease (synchronous or metachronous). For metastatic patients, actionable molecular alterations were identified in 95 patients (43.5%) and for 13 patients (4.7%) a molecularly matched therapy was administered. There was no significant difference in median overall survival between patients who received matched therapy than patients who did not receive molecularly matched therapy (2.89 [95%CI 1.84 - 3.93] vs. 2.86 [95%CI 1.52 - 4.19], p=0.671).

Conclusion: This study suggests that high-throughput genomics can improve management of patients. Although these results did not show a benefit in overall survival for tumors who harboured such actionable molecular alterations and who received molecularly matched therapy, than patients who did not receive molecularly matched therapy, they are promising. Randomized trials are needed to confirm that there is a benefit to treating patients with matched therapy based on NGS.

Reference:
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increasing. Therefore, the number of deaths is expected to increase with the continuous aging of the European population [1]. The molecular pathogenesis of cancer is characterized by the successive acquisition of genetic alterations that lead to aberrant activation of proto-oncogenes and inactivation of tumor suppressor genes. The molecular landscape of cancers of the digestive system are becoming well known, but we need to improve the implications of this new knowledge for the therapeutic management of patients [2, 3].

Now, several testing methods to identify genomic alterations are used in routine clinical practice: detection of a mutant or loss of protein expression immunohistochemistry (IHC), search for mutations in the DNA sequence by Polymerase Chain Reaction (PCR), as well as molecular profiling based on Next Generation Sequencing (NGS). The PCR method (the so-called Sanger method) focuses on a region of interest, such as a single gene or a group of genes selected according to the clinical signs collected and the diagnostic hypothesis made by the prescriber. Thanks to NGS sequencing, it is possible to sequence millions of fragments simultaneously. This high-throughput process results in the sequencing of hundreds to thousands of genes at a time, allowing the detection of novel or rare variants [4]. This method also requires less cellular material, and is faster, less expensive and more accurate. This NGS sequencing includes DNA sequencing looking for genomic mutations, but some genes (notably FGFR2 or NTRK) once transcribed, may form fusions with multiple partners for which additional RNA sequencing is required.

Thanks to this technology, new genes associated with cancer and the clinical significance of these genomic alterations in diagnostic, prognostic and therapeutic management were discovered [5]. Today, based on molecular anomalies, some patients benefit from targeted therapy diagnosed by these genomic methods, either routinely or within the framework of clinical trials or ATU (Temporary Authorization for Use). Promising targeted therapy and personalized medicine are making molecular profiling of tumors a priority. The main actionable mutations and their targeted therapies in digestive oncology are summarized in (Figure 1).

The main objective of our study was to determine the benefit of overall survival of these targeted therapies administered on the basis of NGS data systematically performed at the time of digestive cancer management.

Materials and Methods

I Study Design and Patients

In order to identify the impact of systematic targeted sequencing at diagnosis, we retrospectively collected data from all patients who underwent NGS in the digestive oncology department of Timone Hospital, APHM, Marseille, France, between January 2018 and November 2020 for DNA (mutation NGS) and November 2018 to November 2020 for RNA (fusion NGS). Patients eligible for inclusion in our study were those with i) histologically proven digestive cancer in our center with a sequencing performed at diagnosis and for whom ii) a Multidisciplinary Concertation Meeting has been performed at least for the first line of treatment. The patients excluded from our analysis were those who had no follow-up in our center (death before treatment, absence of data in the medical file), had nondigestive cancers (lymphoma, lung, bladder) and had some particular digestive cancers such as GIST, neuroendocrine tumors, in situ cancers.

The follow-up of patients started at diagnosis with systematic molecular profiling and decision during an RCP (Reunion de concertation
pluridisciplinaire) of a first line of treatment. Tumor samples were collected by endoscopic biopsy or surgical sampling and could be from primary or secondary lesions. These samples were analysed by the Ion Torrent S5 XL ThermoFisher technology, which is an amplicon-based technique, after signing a written and informed authorization. This technique is based on semiconductor chips, filled with wells, which release a proton when a nucleotide is incorporated by the DNA polymerase. This reaction causes a local pH change that is detected by sensors on the chip and converted into raw data in the form of an ionogram, before being transformed into sequence data. Patient data were collected from the computerized medical record of our institution, in particular from the minutes of the multidisciplinary consultation meeting for clinical data and the molecular biology report for mutations.

II Molecular Sequencing

Searches were guided using a validated panel called Oncomine Solid Tumor (OST and OST+) with 22 genes for DNA research: EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKTI, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53; 4 genes for RNA research: ALK, RET, ROS1, NTRK1; and addition of an IDH1 mutation research for advanced biliary cancers, a somatic BRCA mutation research for pancreatic cancer and MSI status by PCR pentaplex [6]. Specific treatments were proposed by the oncologist and discussed in a multidisciplinary consultation meeting, then explained to the patients (inclusion in a trial, ATU or exceptional treatment) who had the choice of accepting or not, after clear information during a dedicated consultation.

Apart from RAS mutations in colorectal cancer and HER2 amplification in gastric cancer, which are already investigated and necessary for therapeutic decision, the actionable mutations retained, according to the literature microsatellite instabilities, mutations affecting the DNA polymerase POLE proofreading domain in colorectal cancer, BRAF mutations, in particular V600E in colorectal cancer, cholangiocarcinoma or pancreatic cancer, ERBB2/HER2 mutations in colorectal cancer or cholangiocarcinoma, MET amplification in colorectal cancer, ALK, ROS1 rearrangements and EGFR mutations in colorectal and pancreatic cancer, NTRK1/3 fusions, BRCA1/2 or even PALB2, ATM, RAD51 mutations, IDH1 and FGFR1/2 fusions or rearrangements in cholangiocarcinoma, KRAS G12C mutations, and more broadly and non-exhaustively thanks to the ‘basket’ clinical trials: PTEN loss or mutations in PIK3CA, AKT1, VEGFR, mTOR pathway (PDGFRA, KIT, DDR1/2), RET fusions or mutations [7-29].

III Outcomes

The primary objective of our single-center retrospective study was to evaluate the overall survival of patients who received decided and targeted therapy on initial NGS data, regardless of the treatment line. We performed exploratory indirect comparisons against patients who received standard lines of therapy and against literature data. Patients were followed throughout their treatment at our center. We constituted two separate analyses of the overall survival of patients with metastatic cancer and those with cancer that remained localized throughout the management. In the metastatic group, we evaluated the overall survival of patients with a ‘hypermutated’ mutation status (with at least 4 mutations found) and those without a hypermutated or mutation status, as well as survival by organ and by mutation.

IV Statistical Analyses

To compare proportions (e.g., MSI status) a χ² test was used for categorical variables and a Mann-Whitney test for ordinal variables. We performed survival analyses using the Kaplan-Meier method and compared the curves with a log-Rank test; estimates are reported for the medians with 95% confidence intervals. A significance level for p values was set at 0.05 for all statistical tests. All the analyses were performed with IBM SPSS Statistics 26.0 (IBM Inc., New York, USA).

Results

Of the 535 patients who had NGS sequencing requested by a Timone APHM digestive functional unit between January 1, 2018, and November 30, 2020, 461 were included in our study (Figure 2). Among the patients excluded from the analysis, 21 patients (3.9%) had GIST or NET, and were therefore excluded from our analysis due to significant differences in management and tumor oncogenesis of these particular tumor types. Fifteen patients (2.8%) had a digestive location of a non-digestive primary (melanoma, bladder cancer, lymphoma, pleural mesothelioma, lung cancer). Two patients (0.4%) died before any medical management. Eight patients (1.5%) had been sequenced on surgical specimens or biopsies, but the histological analysis did not reveal any carcinomatous proliferation. Four patients (0.7%) had no clinical data available and twenty-four (4.5%) were followed up in another center.

![Figure 2: Flow Chart.](image)

Patient Characteristics

The characteristics of the patients are presented in (Table 1). For the 461 patients included, the mean age at diagnosis was 66.2 years (extremes 17.5 - 95.6 years), 281 were male (61%). The most frequent tumor type was colorectal cancer (n=248, 53.8%), ahead of the other tumor locations: pancreatic cancer (n=67, 14.5%), cholangiocarcinoma (n=54, 11.7%), stomach cancer (n=45, 9.8%), esophageal cancer (n=21, 4.6%) and other minority localizations (anus, n=10, 2.2%; Peritoneum, n=6, 1.3%; Gallbladder, n=5, 1.1% and of unknown primary, n=5, 1.1%). 275 patients were metastatic (60.7%), 161 synchronous (34.9%) and 114 metachronous (24.7%), 186 patients did not develop metastases (40.3%).
### Table 1: Patient characteristics.

|                                | Cohort analysis (N=461) | Metastatic (synchronous and metachronous), (N=275) |
|--------------------------------|-------------------------|-----------------------------------------------|
| **Sex**                        |                         |                                               |
| Male                           | 281 (61%)               | 173 (62.9%)                                   |
| Female                         | 180 (39%)               | 102 (37.1%)                                   |
| **Age at diagnosis, years**    |                         |                                               |
| ≤ 65 years                     | 203 (44%)               | 129 (46.9%)                                   |
| > 65 years                     | 258 (56%)               | 146 (53.1%)                                   |
| Mean (range)                   | 66.2 (17.5 – 95.6)      | 65.2 (19.5 – 90.2)                            |
| **Tumor localization**         |                         |                                               |
| Esophagus                      | 21 (4.6%)               | 11 (4%)                                       |
| Stomach                        | 45 (9.8%)               | 32 (11.6%)                                    |
| Colon - Rectum                 | 248 (53.8%)             | 130 (47.3%)                                   |
| Pancreas                       | 67 (14.5%)              | 49 (17.8%)                                    |
| Biliary tract                  | 54 (11.7%)              | 35 (12.7%)                                    |
| Other (Anus, Grèle, Péritoine, Primitif inconnu) | 26 (5.6%)              | 18 (6.5%)                                    |
| **Localized**                  | 186 (40.3%)             | /                                             |
| Synchronous metastases         | 161 (34.9%)             | 161 (58.5%)                                   |
| Metachronous metastases        | 114 (24.7%)             | 114 (41.5%)                                   |
| **Histological type**          |                         |                                               |
| ADK                            | 435 (94.4%)             | 256 (93.1%)                                   |
| SCC                            | 17 (3.7%)               | 10 (3.6%)                                     |
| Undifferentiated carcinoma     | 9 (2%)                  | 9 (3.3%)                                      |
| **MSS**                        | 401 (87%)               | 247 (89.8%)                                   |
| MSI                            | 42 (9.1%)               | 17 (6.2%)                                     |
| No available                   | 18 (3.9%)               | 11 (4%)                                       |
| **Mutation found**             |                         |                                               |
| No                             | 70 (15%)                | 45 (16.4%)                                    |
| Yes                            | 391 (84.8%)             | 230 (83.6%)                                   |
| **Fusion found**               |                         |                                               |
| No                             | 306 (66.4%)             | 188 (68.4%)                                   |
| Yes                            | 302 (98.7%)             | 186 (98.9%)                                   |
| 4 (1.3%)                       |                         | 2 (1.1%)                                      |
| **Number of mutations**        |                         |                                               |
| 0                              | 72 (15.6%)              | 47 (17.1%)                                    |
| < 4                            | 360 (78.1%)             | 211 (76.7%)                                   |
| ≥ 4                            | 29 (6.3%)               | 17 (6.2%)                                     |
| **Targeted therapy**           |                         |                                               |
| Alive                          | 228 (49.5%)             | 121 (44%)                                     |
| Dead                           | 143 (31%)               | 108 (39.3%)                                   |
| Loss of follow-up              | 90 (19.5%)              | 46 (16.7%)                                    |

### Table 2: Mutations found with NGS.

|                                | Cohort analysis (N=461) | Metastatic (synchronous and metachronous), (N=275) |
|--------------------------------|-------------------------|-----------------------------------------------|
| **MSS**                        |                         |                                               |
| MSI                            | 401 (87%)               | 247 (89.8%)                                   |
| No available                   | 42 (9.1%)               | 17 (6.2%)                                     |
| Yes                            | 18 (3.9%)               | 11 (4%)                                       |
| **Mutation RAS**               |                         |                                               |
| No                             | 250 (54.2%)             | 150 (54.5%)                                   |
| Yes                            | 211 (45.8%)             | 125 (45.5%)                                   |
| including G12C                 | 7 (1.5%)                | 6 (2.2%)                                      |
| **Mutation BRAF**              |                         |                                               |
| No                             | 422 (91.5%)             | 254 (92.4%)                                   |
| Yes                            | 39 (8.5%)               | 21 (7.6%)                                     |
Targeted molecular DNA sequencing (NGS Mutation) was routinely performed at diagnosis for all included patients (n=461) and RNA sequencing (so-called NGS Fusion) was performed in those included as of November 2018 (n=306, or 66.4%). Genomic reports were validated by a molecular geneticist. The therapeutic interest of the target was defined in multidisciplinary consultation meeting by the referring oncologist on the data of the literature. Three hundred and eighty-nine patients harboured at least one mutation (84.3%); a fusion was found in only 4 patients (1.3%). The mutational profiles were varied and are reported in (Figure 3) and (Table 2). In the metastatic population, the frequency of mutations was 52.4% for **TP53**, 45.5% for **RAS**, 9.8% for **SMAD4**, 9.5% for **PIK3CA**, 7.6% for **BRAF**, 6.2% for **MSI**, 3.3% for **PTEN**, 2.9% for **HER2**, 1.1% for **POLE**, and <1% for the other mutations. One hundred and ninety-nine actionable molecular targets were detected (199/461, or 43.2%) and 95 in metastatic patients (95/275, or 34.5%).

In our study 13/275 (4.7%) patients were eligible for targeted therapy on initial NGS results, 8 for colorectal cancer (5 MSI, 1 **POLE**, 1 **ATR**, and 1 **PIK3CA**), 1 for gastric cancer (**POLE**), 1 for pancreatic cancer (MSI), and 3 for cholangiocarcinoma (2MSI and 1 **IDH1**), (Table 3). There was no significant difference in survival related to the use of targeted therapy on initial NGS data in metastatic versus non-metastatic patients (2.89 [95%CI 1.84 - 3.93] versus 2.86 [95%CI 1.52 - 4.19], p=0.671) (Figure 4).

| Mutation | No | Yes | No | Yes |
|----------|----|-----|----|-----|
| **PIK3CA** | 400 (86.8%) | 61 (13.2%) | 249 (90.5%) | 26 (9.5%) |
| **HER2** | 449 (97.4%) | 12 (2.6%) | 267 (97.1%) | 8 (2.9%) |
| **EXCEPT OESOGASTRIQUE** | 3 (0.7%) | 1 (0.4%) | 3 (0.7%) | 1 (0.4%) |
| **TP53** | 220 (47.7%) | 47 (10.2%) | 248 (90.2%) | 27 (9.8%) |
| **SMA4D** | 414 (89.8%) | 1 (0.2%) | 47 (10.2%) | 23 (5.8%) |
| **AKT1** | 6 (1.3%) | 1 (0.4%) | 3 (0.7%) | 2 (0.7%) |
| **PDGFRa** | 4 (0.9%) | 1 (0.4%) | 11 (2.4%) | 9 (3.3%) |
| **PTEN** | 2 (0.4%) | 1 (0.4%) | 2 (0.4%) | / |
| **REL** | 2 (0.4%) | 1 (0.4%) | 2 (0.4%) | / |
| **BRCA** | 1 (0.2%) | 1 (0.2%) | 1 (0.2%) | 1 (0.2%) |
| **DDR2** | 5 (1.1%) | 1 (0.4%) | 3 (0.7%) | 2 (0.7%) |
| **FGFR 1/2** | 4 (0.9%) | 1 (0.4%) | 2 (0.7%) | / |
| **PALB2** | 1 (0.2%) | 1 (0.4%) | 1 (0.2%) | 1 (0.4%) |
| **Fusion** | N=306 | N=188 |
| **ALK** | 2 (0.7%) | 1 (0.5%) |
| **ROS1** | 1 (0.3%) | 1 (0.5%) |
| **RET** | 1 (0.3%) | / |
| **Other mutations** | | | | |
| 1 | 43 (9.3%) | 19 (6.9%) |
| 2 | 10 (2.2%) | 8 (2.9%) |
| 3 | 1 (0.3%) | 1 (0.5%) |

*Other mutations: Amplification **FGFR1/2**, Amplification **MYC**, Mutation **CHECK2**, Mutation **CDK 12**, Mutation **CCND1**, Mutation **CTNNB1**, Mutation **ERBB4**, Mutation **FBXW7**, Mutation **FGFR3**, Mutation **STK11**, Mutation **APC**, Mutation **KEAP1**.
Figure 3: Mutations found with NGS

Figure 4: Kaplan-Meier curve of Overall Survival for metastatic patients using targeted therapy (n=13) versus no targeted therapy (n=262), primary objective.
Table 3: Characteristics of patients treated with targeted therapy.

| Organ | Target | Treatment | Type | Treatment line | Duration of treatment | Best Response | Follow-up (year) |
|-------|--------|-----------|------|----------------|-----------------------|--------------|-----------------|
| Colon | MSI    | Nivolumab | Clinical trial, phase 3 | L2 | Stable disease at 1 month then progression at 3 months | 1 month | 2.84 |
|       | ATR    | AZD6738 (ATR COMBO) | Clinical trial, phase 1 | L3 | Progression at 1 months | NA | 4.2 |
| MSI   | Pembrolizumab | Compassionate, out of AMM | L2 | Doubt about pseudo progression at 4 months then confirmed progression at 5 months | 4 months | 2.51 |
| MSI   | Nivolumab – Ipilimumab | Clinical trial, phase 3 | L2 | Stable disease at 1 month then progression at 3 months | 10 months | 2.16 |
| POLE  | Nivolumab | Clinical trial, phase 3 | L2 | Objective response at 5 months and progression at 7 months | 5 months | 1.38 |
| PIK3C A | Aspirin | Out of AMM | L1 | Objective response at 12 months | 12 months | 3.61 |
| MSI   | Pembrolizumab | Compassionate, out of AMM | L1 | Progression at 1 months | NA | 0.34 |
| MSI   | Nivolumab | Clinical trial, phase 3 | L1 | Objective response at 5 months, treatment ongoing | 5 months | 0.52 |
| Stomach | POLE | Nivolumab | Clinical trial, phase 3 | L3 | Pseudo progression at 1 month and confirmed progression at 3 months | NA | 2.16 |
| Pancreas | MSI | Pembrolizumab | Compassionate, out of AMM | L4 | Progression at 1 month | NA | 2.86 |
| Biliary tract | MSI | Atezolizumab | Compassionate, out of AMM | L2 | Stable at 3 months | 3 months | 2 |
| IDHI | FT-2102 | Clinical trial, phase 1b/2 | L2 | Objective response at 8 months and progression at 13 months | 8 months | 2.61 |
| MSI   | Nivolumab | Clinical trial, phase 3 | L2 | Objective response at 34 months (treatment duration 24 months) | 34 months | 3.73 |

In the general population, the median overall survival was 4.19 years [95%IC 2.78 - 5.61] (Figure 5A). In patients who did not develop metastases, the median survival was not reached (Figure 5B). In the metastatic population (synchronous or metachronous, n=275, 60.7%), the median overall survival at diagnosis of cancer disease was 4.13 years [95%IC 3.04 - 5.21], with a median of 2.66 years [95%IC 2.03 - 3.29], the presence of synchronous metastases was associated with worse survival at diagnosis than their metachronous occurrence (5.69 years, [95%IC 2.57 - 8.81], p < 0.0001).

Figure 5: Kaplan-Meier curve of Overall Survival at diagnosis for metastatic patients (n=275), metachronous (n=114) or synchronous (n=161), versus no metastatic (n=186), after sequencing.

The median overall survival was 2.86 years [95%IC 1.84 - 3.87] from diagnosis of metastatic disease (Figure 6A). There was no significant difference in survival by gender (Male 2.23 [95%IC 0.85 - 3.61] versus Female 3.17 [95%IC 2.12 - 4.22], p=0.683) and age (≤65 years 3.17 [95%IC 2.09 - 4.26] versus >65 years 2.23 [95%IC 0.96 - 3.51], p=0.332). There was no difference in survival by mutational status, < 4 mutations or ≥ 4 mutations (2.66 [95%IC 1.65 - 3.68] versus 4.19 [95%IC 1.86 - 6.53], p=0.681) in metastatic population and by organ subgroup, but non-mutated patients had a median survival of 2.17 years [95%IC 1.62 - 2.71] which appeared to be lower than "hypermutated" patients although not significant (4.19, [95%IC 1.86 - 6.53], p=0.792), (Figures 6C & 6D).
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Figure 6: Kaplan-Meier curve of Overall Survival for metastatic patients (n=275) after diagnosis of A) metastases, B) per organ, and C & D) according to the number of mutations.

Figure 7: Kaplan-Meier curve of overall survival, according to MSI status (MSI, n=30 or MSS, n=211) in the colorectal cancer subgroup [A] Non metastatic, B) Metastatic] and in the general population by mutation number.

We also compared survival by organ and mutation type (Figure 6B). MSI status was significantly more frequently associated with non-metastatic disease (Odds Ratio 0.42 [0.22 - 0.8], p=0.008) as well as with a ‘hypermutated’ status with a non-homogeneous distribution according to the number of mutations (p=0.043) (Figure 7).

In the localized pancreatic cancer subgroup, there was a difference in survival according to TP53 status in favour of mutated patients with a median not reached (p=0.043), which was not found in metastatic patients. On the contrary, in metastatic patients, there was a difference in survival between mutated and wild-type RAS patients, with a better survival in mutated RAS patients (1.44 [95%IC 1.53 - 2.59] in wt RAS patients vs 6.65, p=0.035). (Figure 8).
Figure 8: Overall survival in the pancreatic cancer subgroup according to mutation TP53 [A) no metastatic, and B) metastatic] or mutation RAS [C) no metastatic, D) and metastatic].

Discussion

In our study, no overall survival benefit was found for patients treated with a therapy adapted to a molecular target detected on an NGS systematically performed at the time of medical management. However, there is a large mutational panel of tumors in digestive oncology as shown in this study. Indeed, 82.9% of patients have at least one mutation in the metastatic situation, and many targets potentially actionable outside the current marketing authorization were found (43.2% in the overall population and 34.5% in the metastatic situation). Our study is large, with a large number of patients, collected in real life and with a follow-up of nearly 3 years, and descriptive data on the mutational landscape of digestive cancers provided on a large database for future studies. Moreover, the tumor locations were diversified with almost 50% of non-colorectal cancers, accounting for differences in oncogenesis between the different tumor subtypes and may allow the selection of patients for future trials targeting a specific molecular alteration. We also included patients without limiting ourselves to metastatic patients, giving us an idea of the temporal dynamics of genomic alterations. Some patients showed a ‘hypermutated’ status, which seems to predict a response to immunotherapy, in the KEYNOTE 028 trial as well as in the preliminary results of the TAPUR trial and thus could allow the indication for immunotherapy to be extended, especially in those without microsatellite instability [30, 31].

This study, although based on overall survival which is a strong and relevant assessment, is limited by the fact that it is retrospective. Indeed, some patients included at the beginning of the study showed mutations that had not yet been identified as potentially actionable and could have been the subject of targeted treatment if they had been discovered at the end of the study. Thus, despite the duration of nearly 3 years, which may seem relatively short, the targeted therapies proposed in 2020 were more numerous than those existing in 2018. Regarding the main objective of the benefit of targeted therapy on overall survival, the results obtained in this study cannot be generalized to each tumor subgroup because of the heterogeneity of our cohort. Indeed, each tumor subgroup is not impacted in the same way by the implementation of a targeted treatment on a mutation found by NGS. This tumor heterogeneity would lead to a lack of power if we were to analyse the benefit of a targeted therapy based on each location.

There has long been controversy about whether the use of genomics could improve survival outcomes in patients with difficult-to-treat cancers. Despite an early negative study, recent studies now show a clear survival benefit from searching for and treating activatable tumor abnormalities, with prolonged survival in subgroups and sometimes clinical and objective responses [32]. Schwaederle et al. conducted meta-analyses of phase 1 and phase 2 trials of a personalized oncology treatment strategy targeting any biomarker, and in both cases demonstrated that the use of molecularly targeted therapy resulted in a higher response rate and improved progression-free survival and overall survival compared with standard therapy [33, 34]. In the IMPACT/COMPACT trial, conducted by Stockley et al. 1893 patients were enrolled and 1640 were tested. 84 patients were treated with molecularly targeted therapy (5%) with a significantly higher overall response rate [35]. In the WINTHER trial, 107 patients received molecularly targeted therapy based on DNA sequencing (n=69) or RNA sequencing (n=38), the rate of stabilized disease > 6 months, partial response or complete response was 26.2% [36]. These results were also found to a greater extent in the MOSCATO-01 trial conducted by Massard et al. where tumor sequencing improved survival for 33% of patients with advanced cancers, particularly in advanced biliary cancers [37, 38]. This benefit was also found in the Pishvaian et al. trial in metastatic pancreatic cancer, with a median survival of up to 2.58 years.
in the group treated with molecularly targeted therapy, and no other therapeutic modality offers a benefit of this magnitude in this patient population [39].

The realization of a systematic NGS at diagnosis is an important question today. At the economic level in France, this analysis is not yet part of the social security acts. It is registered in the list of acts outside the nomenclature (RIHN), with a cost of 882.90 euros per sequencing, that is to say 1765.80 euros for a DNA and RNA research. The hospital is reimbursed at about 50%. In addition, logistical issues and the aggressiveness of the disease are also factors to be taken into account. In our study, we performed this sequencing at diagnosis for all patients regardless of their disease status and this may have caused an important selection bias, 7 patients died less than one month after their sequencing, 186 patients remained localized. Limiting the prescription of NGS to patients of oncological interest, to assess the tumor and dynamic genetic heterogeneity, which is at the origin of the secondary resistance to treatments. In a trial presented at ASCO in 2014 by Lim et al., the results of full tumor sequencing were compared with targeted sequencing (AmpliSeq panel) on 56 patients with advanced breast, lung, and colorectal cancers. Whole genome sequencing was more informative than targeted sequencing (70% of cases compared with 30% of cases) [41]. However, in the MOSCATO-01 trial, whole exome sequencing detected mutations in 8 patients who had no alterations on targeted sequencing and in situ hybridization tests, which represents less than 10% of the patients included and suggests that targeted sequencing can ensure detection of the most common alterations (Table 4) [37, 42].

Table 4: Main clinical trial about NGS and targeted therapy.

| Clinical Trial | Center | Date | Cancer type | Sequencing method | Number of patients | Targeted therapy | Response |
|----------------|--------|------|-------------|-------------------|-------------------|------------------|----------|
| Le Tourneau et al., Lancet Oncol, 2015 [32] | SHIVA trial | Multicenter, 5 centers (France) | October 2012 to July 2014 | Advanced solid tumor | DNA sequencing by AmpliSeq | 741 patients screened, 293 with at least one molecular alteration (40%) | 195 (26%) patients had been randomly assigned, with 99 for matched molecularly targeted agent and 96 for treatment at physician’s choice | Progression Free survival 2.3 months in the experimental subgroup [95%IC 1.7 – 3.8] vs 2 months [95%IC 1.8 – 2.1], HR 0.88, p=0.41 |
| Rodon et al, Nat Med, 2019 [36] | WINThER trial | Multicenter, 5 centers (France, Espagne, Canada, Etats Unis) | April 2013 to December 2015 | Colon, Head and neck, Lung cancers and other advanced solid tumors. | DNA sequencing with a panel of 236 genes and RNA expression transcriptome | 303 patients consented, 253 tested | 107 patients evaluable for therapy (35%) | The rate of stable disease > 6 months and partial or complete response was 26.2% |
| Stockley et al, Genome med, 2016 [35] | IMPACT / COMPACT trial | Monocenters Princess Margarette Cancer Center, Ontario, USA | March 2012 to July 2014 | Advanced solid tumors. | MALDI TOF Hotspot panel and Targeted NGS panel. | 1893 patients included, 1640 tested | 89 patients treated with target therapy (5%) | Overall response rate higher in patients treated on genotype-matched trials (19%) versus genotype-unmatched trials (9%, p<0.026) |
| Massard et al., Cancer Discov, 2017 [37] | MOSCATO-01 trial | Monocenter (Institute Gustave Roussy, Paris – France) | December 2011 to March 2016 | Advanced solid tumors | DNA sequencing by AmpliSeq – ThermoFisher and array comparative genomic hybridization | 1035 patients included, 843 tested (89%) and 411 with a molecular target alteration (49%) | 199 patients were treated with a targeted therapy matched to a genomic alteration. | The PFS2/PFS1 ratio was >1.3 in 33% of the patients (63/193). Objective responses were observed in 22 of 194 patients (11%; 95% CI, 7%–17%), and median overall progression-free survival (PFS) was 14.6 months vs 10.2 months in the experimental subgroup vs 2 months (95%CI 1.8 – 2.1), HR 0.88, p=0.41 |

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High-throughput sequencing technologies are not part of international recommendations, although they are performed on a clinical routine basis in many centers, and similar observational trials are appearing, such as the study conducted in Paris by the team of Bayle et al. [43]. Prospective multi-center studies on larger series are considered and will allow a more significant analysis of the impact of precision medicine on patient prognosis. The current challenge, supported by learned societies and the France Genomics 2025 Plan, is to improve the effectiveness of precision medicine, its indications, its accessibility and its use, in order to extend the number of patients treated and have an impact on survival.

**Conclusion**

Although our study did not find an overall survival impact of using a targeted therapy on an actionable molecular abnormality, the mutational panel of digestive cancers is large, and these results pave the way for the future of prospective clinical trials guided by molecular profiling. Only prospective, therapeutic interventional trials by molecular or tumor alteration subgroups will definitively demonstrate the benefits of these therapies and are the next logical step in clinical trials for patients with digestive cancer.

**Ethical Approval**

The study was conducted in accordance with the good clinical practices of the International Conference on Harmonization and the recommendations of the Declaration of Helsinki.

**Informed Consent**

Written informed consent was obtained from all for the DNA/RNA sequencing analysis.

**Conflicts of Interest**

None.

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