Available online at www.sciencerepository.org

Science Repository

Research Article

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

Bernadette de Rauglaudre[1*], Emmanuelle Norguet-Monnereau[1], Muriel Duluc[1], Isabelle Nanni[2], Julien Mancini[3] and Laetitia Dahan[1]

[1]Assistance Publique, Hôpitaux de Marseille, Department of Digestive Oncology, CHU Timone, Aix-Marseille University, Marseille, France
[2]Assistance Publique, Hôpitaux de Marseille, Department of Molecular Oncology, CHU Nord, Aix-Marseille University, Marseille, France
[3]Assistance Publique, Hôpitaux de Marseille, Department of Public Health and Medical Information, CHU Timone, Aix-Marseille University, Marseille, France

ARTICLE INFO

Article history:
Received: 25 June, 2021
Accepted: 12 July, 2021
Published: 27 July, 2021

Keywords:
High-throughput molecular sequencing
digestive oncology
mutation
targeted therapy

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 in95 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.

Introduction

There were an estimated 3.9 million new cancer cases and 1.9 million deaths from cancer in Europe in 2018 excluding non-melanoma skin cancer). Digestive cancers represent a quarter of the overall incidence (Esophagus 1.4%, Stomach 3.4%, Colorectal 12.8%, Liver 2.1%, Pancreas 3.4%, i.e., 23.1%) and a third of cancer mortality (Esophagus 2.3%, Stomach 5.3%, Colorectal 12.6%, Liver 4%, Pancreas 6.6%, i.e., 30.8%) [1]. Among these, colorectal cancer is the second leading cause of cancer death in males and the third in females. Pancreatic cancer, whose incidence is increasing, is the fourth cause. The observed trends in digestive cancer mortality rates in Europe are stable or slightly

Correspondence to: Dr. Bernadette de Rauglaudre, Assistance Publique, Hôpitaux de Marseille, Department of Digestive Oncology, CHU Timone, 264, rue saint Pierre, F-13385, Marseille Cedex 05, Aix-Marseille University, Marseille, France; E-mail: Bernadette.rauglaudre@hotmail.fr

http://dx.doi.org/10.31487/j.COR.2021.07.04

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).

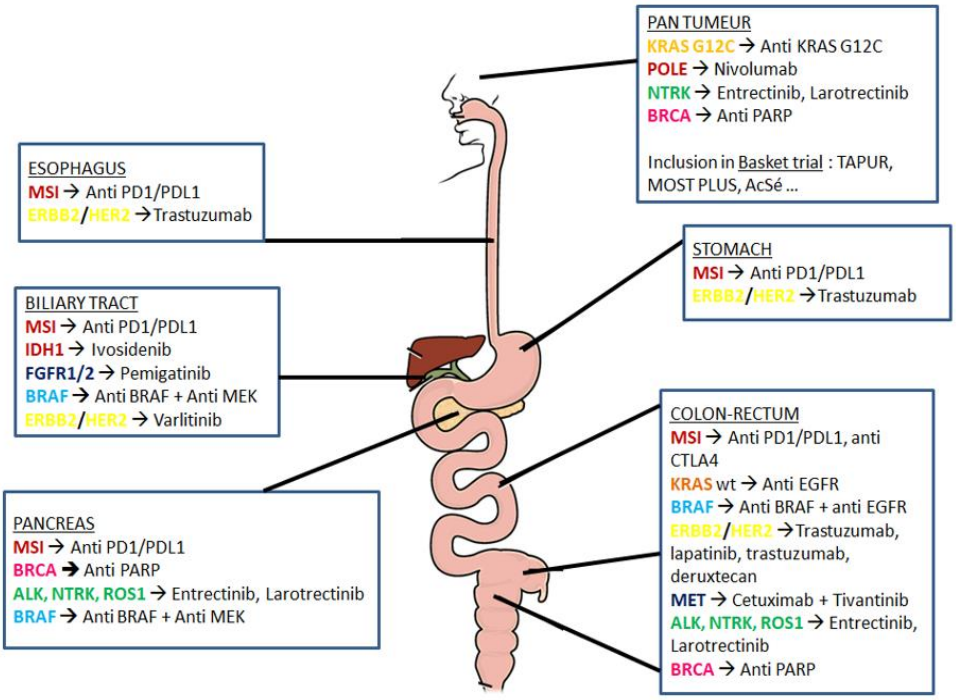


Figure 1: Main actionable molecular alteration according to location.

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, AKT1, 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 cancers 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 χ^2 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.

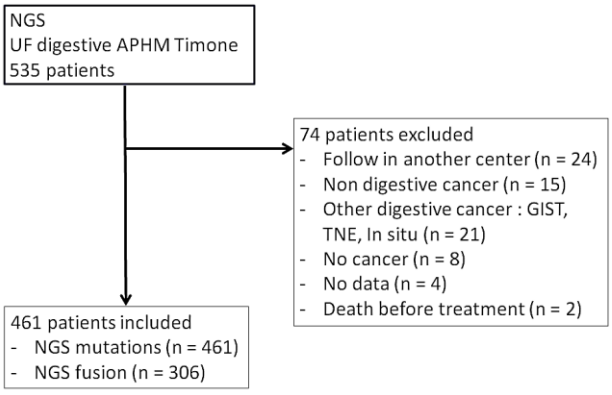


Figure 2: Flow Chart.

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	306 (66.4%)	188 (68.4%)
No	302 (98.7%)	186 (98.9%)
Yes	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	14 (3%)	13 (4.7%)
Patient's condition at the last news		
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	401 (87%)	247 (89.8)
MSI	42 (9.1%)	17 (6.2%)
No available	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%)

including V600E	30 (6.5%)	14 (5.1%)
PIK3CA		
No	400 (86.8%)	249 (90.5%)
Yes	61 (13.2%)	26 (9.5%)
HER 2		
No	449 (97.4%)	267 (97.1%)
Yes	12 (2.6%)	8 (2.9%)
Except Oesogastrique	3 (0.7%)	1 (0.4%)
TP53		
No	220 (47.7%)	131 (47.6%)
Yes	241 (52.3%)	144 (52.4%)
SMA4D		
No	414 (89.8%)	248 (90.2%)
Yes	47 (10.2%)	27 (9.8%)
AKT1	6 (1.3%)	1 (0.4%)
PDGFRA	3 (0.7%)	2 (0.7%)
POLE	4 (0.9%)	3 (1.1%)
PTEN	11 (2.4%)	9 (3.3%)
RET	2 (0.4%)	1 (0.4%)
ALK	2 (0.4%)	/
ATM	2 (0.4%)	2 (0.7%)
BRCA	1 (0.2%)	1 (0.4%)
DDR2	5 (1.1%)	/
EGFR	1 (0.2%)	1 (0.4%)
KIT	4 (0.9%)	2 (0.7%)
IDH1	3 (0.7%)	2 (0.7%)
MET	4 (0.9%)	2 (0.7%)
FGFR 1/2	4 (0.9%)	2 (0.7%)
PALB2	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*.

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).

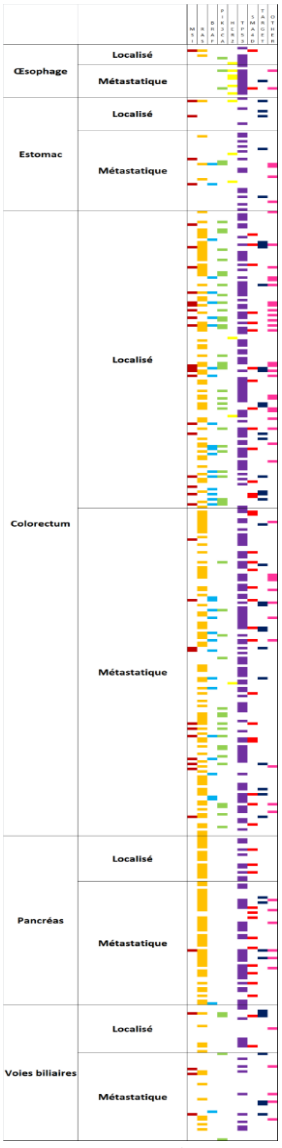


Figure 3: Mutations found with NGS

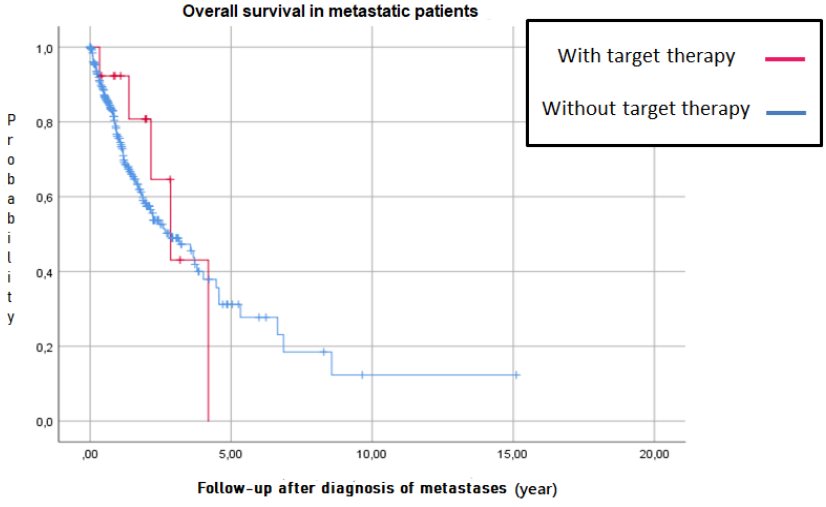


Figure 4: Kaplan-Meier curve of Overall Survival for metastatic patients using targeted therapy (n=13) versus no targeted therapy (n=262), primary objective.

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 I	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
	PIK3CA	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
	IDH1	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).

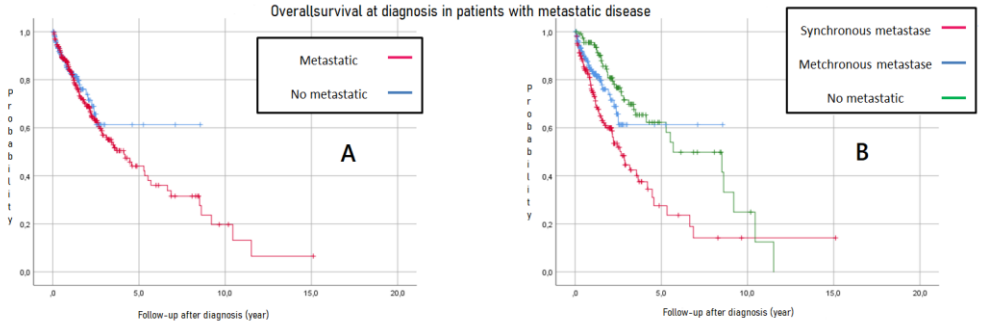


Figure 5: Kaplan-Meier curve of Overall Survival at diagnosis for metastatic patients (n=275), metachronous (n=114) or synchronous (n=161), versus no metastatic (n=186), after sequencing.

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).

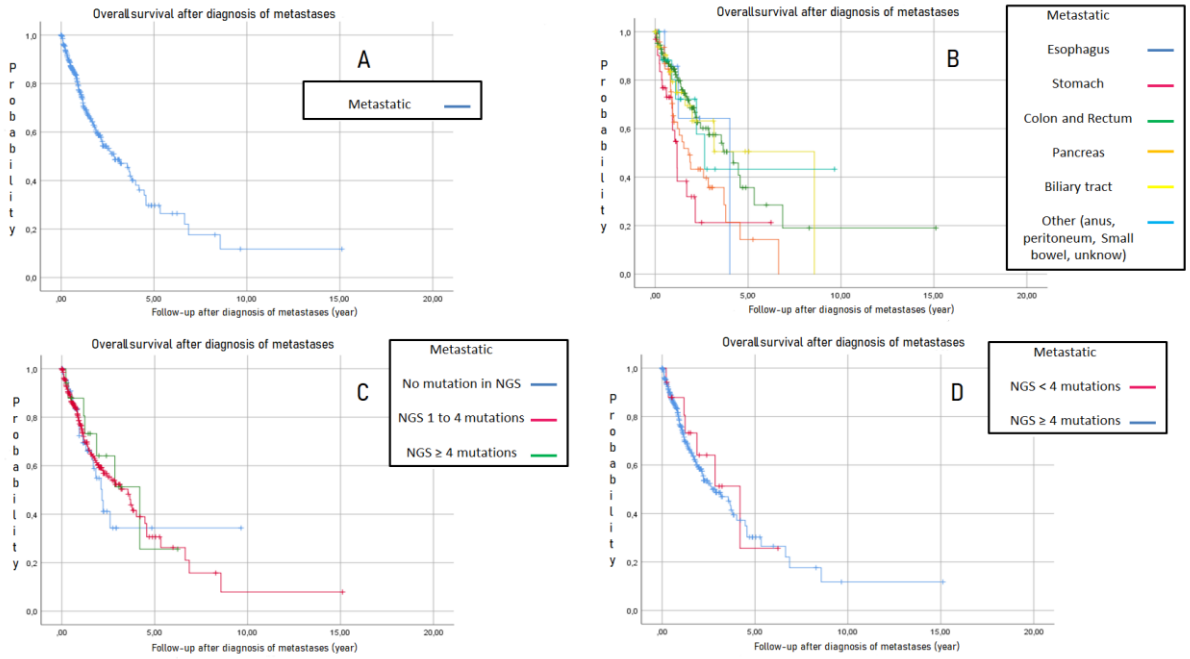
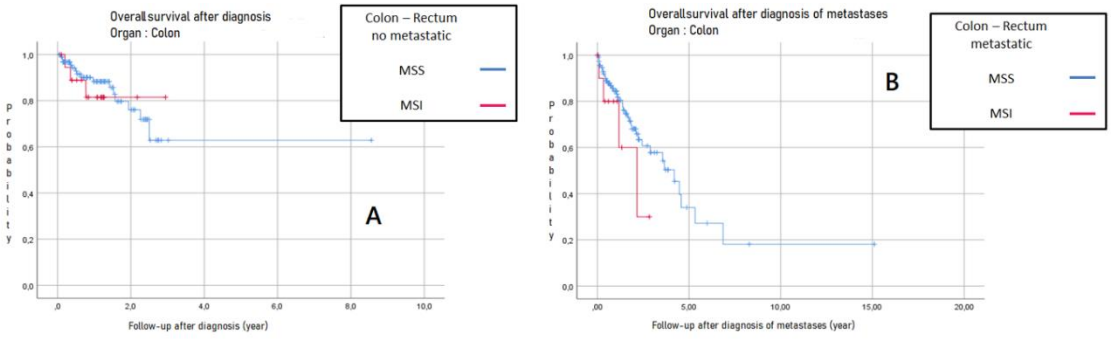


Figure 6: Kaplan-Meier curve of Overall Survival for metastatic patients (n=275) after diagnosis of **A)** metastases, **B)** per organ, and **C** & **D)** according to the number of mutations.



NGS with MSI status research (n = 443)	MSS (n = 401, 90.5%)	MSI (n = 42, 9.5%)
NUMBER OF MUTATIONS		
No mutation (n = 69)	66 (95.7%)	3 (4.3%)
< 4 mutations (n = 345)	311 (90.1%)	34 (9.9%)
= 4 mutations (n = 29)	24 (82.8%)	5 (17.2%)
Non – homogeneous distribution according to the number of mutations (p= 0.043)		
METASTATIC STATUS		
Non metastatic (n = 179)	154 (86%)	25 (14%)
Metastatic (n = 264)	247 (93.6%)	17 (6.4%)
OR 0.42 [0.22 – 0.8], p = 0.008		

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).

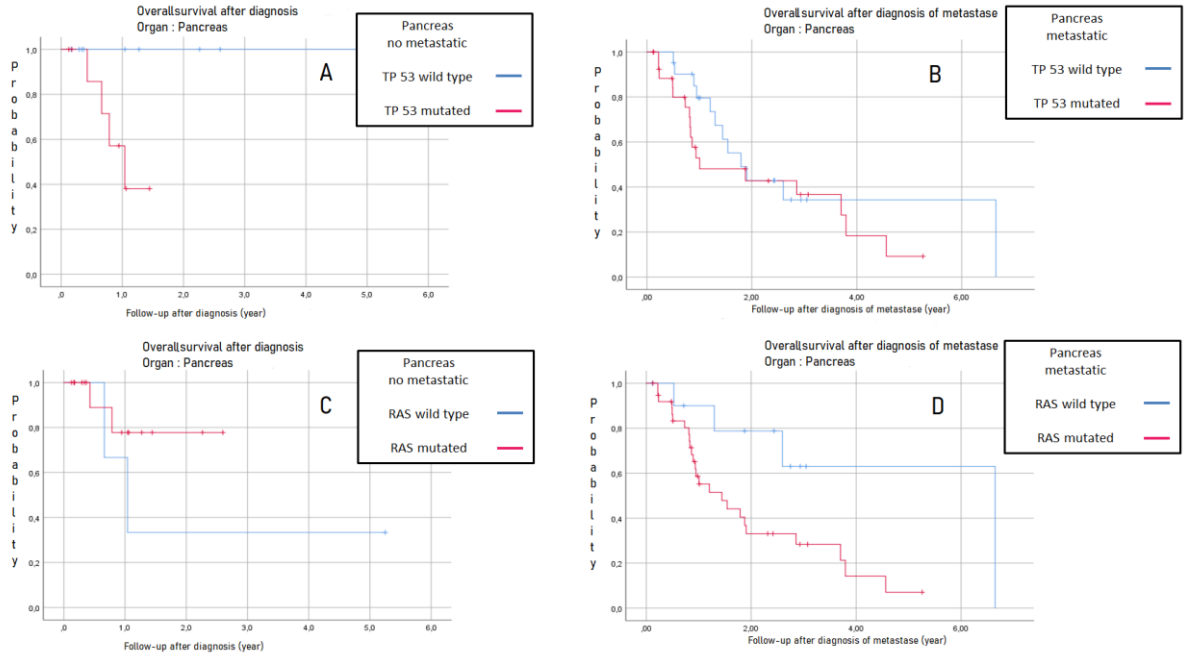


Figure 8: Overall survival in the pancreatic cancer subgroup according to mutation TP53 [**A)** no metastatic, and **B)** metastatic] or mutation RAS [**C)** no metastatic, **D)** and metastatic].

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 metastatic/treatment-refractory situations, or to young patients still able to receive treatment is a key issue [40].

Given the limited number of genes whose analysis is currently of proven medical interest, NGS is currently used in the form of a panel (Targeted sequencing) to rapidly perform an analysis of a selected number of genes of theranostic interest on a large number of patients in order to reduce delays and costs. But an important contribution of the new sequencing technologies in cancer is to have access to the globality of the molecular mechanisms of oncogenesis, by a complete sequencing of the exome (Whole exome sequencing) which enables the identification of molecular profiles and recurrent mutations of nosological, prognostic or theranostic 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

					(aCGH) analysis.			survival was 11.9 months (95% CI, 9.5–14.3 months).
Pishvaian *et al.* The Lancet, 2020 [39]	Retrospective analysis from the KYT program (Know Your Tumor)	Monocenter (Perthera, McLean, USA)	June 2014 to March 2019	Pancreatic adenocarcinoma.	NGS Foundation Onegenes and PGDx R203 Genes. Panel IHC (17 genes) Caris IHC Proteins and NeoGenomics IHC.	1856 patients, 1082 tested (58%), 677 patients for whom outcomes were available	189 had actionable molecular alterations (28%) and 46 received a matched therapy (6.8%)	Median OS 2.58 years [95%IC 2.39 – not reached] versus those patients who only received unmatched therapies, 1.51 years [1.33 – 1.87], HR 0.42, p=0.0004

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.

References

1. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G et al. (2018) Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer* 103: 356-387. [Crossref]
2. Fakhri B, Lim KH (2017) Molecular landscape and sub-classification of gastrointestinal cancers: a review of literature. *J Gastrointest Oncol* 8: 379-386. [Crossref]
3. Stenzinger A, Weichert W (2017) Genetic Profiling of Cancers of the Digestive System: Biological Insights and Clinical Implications. *Pathobiology* 84: 306-322. [Crossref]
4. Rusk N, Kiermer V (2008) Primer: Sequencing--the next generation. *Nat Methods* 5: 15. [Crossref]
5. Fontanges Q, De Mendonca R, Salmon I, Le Mercier M, D'Haene N (2016) Clinical Application of Targeted Next Generation Sequencing for Colorectal Cancers. *Int J Mol Sci* 17: 2117. [Crossref]
6. Williams HL, Walsh K, Diamond A, Oniscu A, Deans ZC (2018) Validation of the Oncomine ™ focus panel for next-generation sequencing of clinical tumour samples. *Virchows Arch* 473: 489-503. [Crossref]
7. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H et al. (2015) PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 372: 2509-2520. [Crossref]
8. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus Acosta A et al. (2020) Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 38: 1-10. [Crossref]
9. Domingo E, Freeman Mills L, Rayner E, Glaire M, Briggs S et al. (2016) Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol* 1: 207-216. [Crossref]
10. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J et al. (2019) Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-Mutated Colorectal Cancer. *N Engl J Med* 381: 1632-1643. [Crossref]
11. Subbiah V, Lassen U, Élez E, Italiano A, Curigliano G et al. (2020) Dabrafenib plus trametinib in patients with BRAF V600E-mutated biliary tract cancer (ROAR): a phase 2, open-label, single-arm, multicentre basket trial. *Lancet Oncol* 21: 1234-1243. [Crossref]
12. Guan M, Bender RJ, Pishvaian MJ, Halverson DC, Tuli R et al. (2018) Molecular and clinical characterization of BRAF mutations in pancreatic ductal adenocarcinomas (PDACs). *J Clin Oncol* 36: 214.
13. Siena S, Di Bartolomeo M, Raghav KPS, Masuishi T, Loupakis F et al. (2020) A phase II, multicenter, open-label study of trastuzumab deruxtecan (T-DXd; DS-8201) in patients (pts) with HER2-expressing

metastatic colorectal cancer (mCRC): DESTINY-CRC01. *J Clin Oncol* 38: 4000.

14. Sartore Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S et al. (2016) Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 17: 738-746. [Crossref]
15. Meric Bernstam F, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M et al. (2019) Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol* 20: 518-530. [Crossref]
16. Javle MM, Oh DY, Ikeda M, Yong WP, McIntyre N et al. (2020) Results from TreeTopp: A randomized phase II study of the efficacy and safety of varlitinib plus capecitabine versus placebo in second-line (2L) advanced or metastatic biliary tract cancer (BTC). *J Clin Oncol* 38: 4597.
17. Rimassa L, Bozzarelli S, Pietrantonio F, Cordio S, Lonardi S et al. (2019) Phase II Study of Tivantinib and Cetuximab in Patients With KRAS Wild-type Metastatic Colorectal Cancer With Acquired Resistance to EGFR Inhibitors and Emergence of MET Overexpression: Lesson Learned for Future Trials With EGFR/MET Dual Inhibition. *Clin Colorectal Cancer* 18: 125.e2-132.e2. [Crossref]
18. Tuli R, Lo S, Koo J, Pishvaian M, Bender RJ et al. (2017) Anaplastic Lymphoma Kinase Rearrangement and Response to Crizotinib in Pancreatic Ductal Adenocarcinoma. *JCO Precis Oncol*.
19. Amatu A, Somaschini A, Cerea G, Bosotti R, Valtorta E et al. (2015) Novel CAD-ALK gene rearrangement is drugable by entrectinib in colorectal cancer. *Br J Cancer* 113: 1730-1734. [Crossref]
20. Doebele RC, Drilon A, Paz Ares L, Siena S, Shaw AT et al. (2020) Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol* 21: 271-282. [Crossref]
21. Kaufman B, Shapira Frommer R, Schmutzler RK, Audeh MW, Friedlander M et al. (2015) Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol* 33: 244-250. [Crossref]
22. Leichman L, Groshen S, O'Neil BH, Messersmith W, Berlin J et al. (2016) Phase II Study of Olaparib (AZD-2281) After Standard Systemic Therapies for Disseminated Colorectal Cancer. *Oncologist* 21: 172-177. [Crossref]
23. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T et al. (2019) Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. *N Engl J Med* 381: 317-327. [Crossref]
24. Abou Alfa GK, Macarulla T, Javle MM, Kelley RK, Lubner SJ et al. (2020) Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol* 21: 796-807. [Crossref]
25. Abou Alfa GK, Sahai V, Hollebecque A, Vaccaro G, Melisi D et al. (2020) Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol* 21: 671-684. [Crossref]
26. Canon J, Rex K, Saiki AY, Mohr C, Cooke K et al. (2019) The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 575: 217-223. [Crossref]
27. American Society of Clinical Oncology (2021) Targeted Agent and Profiling Utilization Registry (TAPUR) Study. *Clinicaltrials.gov*.
28. UNICANCER (2021) Secured Access to Nivolumab for Adult Patients With Selected Rare Cancer Types (AcSé). *Clinicaltrials.gov*.
29. Centre Leon Berard (2021) A Two-period, Multicenter, Randomized, Open-label, Phase II Study Evaluating the Clinical Benefit of a Maintenance Treatment Targeting Tumor Molecular Alterations in Patients With Progressive Locally-advanced or Metastatic Solid Tumors. *Clinicaltrials.gov*.
30. Ott PA, Bang YJ, Piha Paul SA, Razak ARA, Bennouna J et al. (2019) T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. *J Clin Oncol* 37: 318-327. [Crossref]
31. Meiri E, Garrett Mayer E, Halabi S, Mangat PK, Shrestha S et al. (2020) Pembrolizumab (P) in patients (Pts) with colorectal cancer (CRC) with high tumor mutational burden (HTMB): Results from the Targeted Agent and Profiling Utilization Registry (TAPUR) Study. *J Clin Oncol* 38: 133.
32. Le Tourneau C, Delord JP, Gonçalves A, Gavoille C, Dubot C et al. (2015) Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol* 16: 1324-1334. [Crossref]
33. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL et al. (2015) Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol* 33: 3817-3825. [Crossref]
34. Schwaederle M, Zhao M, Lee JJ, Lazar V, Leyland Jones B et al. (2016) Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. *JAMA Oncol* 2: 1452-1459. [Crossref]
35. Stockley TL, Oza AM, Berman HK, Leighl NB, Knox JJ et al. (2016) Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret IMPACT/COMPACT trial. *Genome Med* 8: 109. [Crossref]
36. Rodon J, Soria JC, Berger R, Miller WH, Rubin E et al. (2019) Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nat Med* 25: 751-758. [Crossref]
37. Massard C, Michiels S, Ferté C, Le Deley MC, Lacroix L et al. (2017) High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial. *Cancer Discov* 7: 586-595. [Crossref]
38. Verlingue L, Malka D, Allorant A, Massard C, Ferté C et al. (2017) Precision medicine for patients with advanced biliary tract cancers: An effective strategy within the prospective MOSCATO-01 trial. *Eur J Cancer* 87: 122-130. [Crossref]
39. Pishvaian MJ, Blais EM, Brody JR, Lyons E, DeArbeloa P et al. (2020) Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *Lancet Oncol* 21: 508-518. [Crossref]
40. Colomer R, Mondejar R, Romero Laorden N, Alfranca A, Sanchez Madrid F et al. (2020) When should we order a next generation sequencing test in a patient with cancer? *EClinicalMedicine* 25: 100487. [Crossref]

41. Lim HJ, Shen Y, Laskin JJ, Gelmon KA, Renouf DJ et al. (2014) The use of whole-genome sequencing in therapeutic for decision making in patients with advanced malignancies. *J Clin Oncol* 32: 11026.
42. Postel Vinay S, Boursin Y, Massard C, Hollebecque A, Ileana E et al. (2016) Seeking the driver in tumours with apparent normal molecular profile on comparative genomic hybridization and targeted gene panel sequencing: what is the added value of whole exome sequencing? *Ann Oncol* 27: 344-352. [Crossref]
43. JFHOD | SNFGE.org (2021) Société savante médicale française d'hépato-gastroentérologie et d'oncologie digestive.