Exploring the Role of Immunotherapy-Based Treatments for Advanced Non–Small-Cell Lung Cancer With Novel Driver Alterations

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Abstract

Impact of novel driver alterations on response to immunotherapy (IO) in advanced non–small-cell lung cancer (aNSCLC) is not well understood. In this study IO response of 84 aNSCLC patients harboring novel driver alterations (m-cohort) was analyzed and compared to a wild-type cohort. Overall, no detrimental effect was identified for IO based treatments for m-cohort. Adding chemotherapy could improve outcomes.

Background: Immunotherapy (IO) single agent or combined with chemotherapy (CT-IO) is the standard treatment for advanced non–small-cell lung cancer (aNSCLC) without driver alterations. IO efficacy in patients with novel driver alterations is not well reported.**Materials and Methods:** Data of aNSCLC patients treated with IO or CT-IO in any line from January 2016 to September 2022 were retrospectively collected. Patients harboring novel driver alterations (m-cohort), including MET exon 14 skipping, BRAF (V600E or atypical), RET rearrangements, HER2 point mutations/exon 20 insertions or uncommon EGFR mutations/EGFR exon 20 insertions, and wild type patients (wt-cohort) were eligible. Clinico-pathological data were extracted from Institutional databases and compared through chi square or Fisher's exact test. Survivals were estimated through Kaplan-Meier method and compared by log-rank test. **Results:** m-cohort and

Abbreviations: aNSCLC, advanced non–small-cell lung cancer; anti-CTLA-4, anti-cytotoxic T-lymphocyte-associated protein 4; anti-PD-1, anti-programmed cell death protein 1; anti-PD-L1, anti-programmed cell death protein ligand 1; BMI, body mass index; BRAFat, BRAF atypical mutation; BRAFV600E, BRAF V600E; COSMIC, Catalogue of Somatic Mutations in Cancer; CR, complete respose; CT-IO, immunotherapy combination with platinum chemotherapy; DCR, disease control rate; EGFRex20, EGFR exon20 insertions; HER2ex20, HER2 exon 20 insertions; HER2mut, HER2 point mutations; HR, hazard ratio; IO, immunotherapy single agent; m-follow up, median follow-up; mAbs, monoclonal antibody; METex14, MET exon 14 skipping mutations; mo, months; ORR, overall response rate; PCR, polymerase chain reaction; PD, progressive disease; PFS, progression free survival; PR, partial response; PSM, propensity score matching; RCTs, randomized controlled trials; RECIST, Response Evaluation Criteria in Solid Tumors; RETr, RET rearrangements; SD, stable disease; TA, target agents; TB CT, total body computed tomography; TMB, tumor mutational burden; TME, tumor microenvironment; uEGFR, uncommon EGFR mutations.

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wt-cohort included 84 and 444 patients, respectively. Progression free survival (PFS) was 5.53 vs. 4.57 months (P= .846) and overall survival (OS) was 25.1 vs. 9.37 months, ($P < .0001$) for m-cohort compared to wt-cohort. Within the m-cohort, BRAF atypical mutations had the better outcomes (Overall Response Rate [ORR], PFS), targeted agents timing did not affect response to IO and CT-IO had better ORR and disease control rate (DCR) compared to IO single agent ($P = .0160$ and $P = .0152$). In the PD-L1≥50% group, first line IO single agent resulted in inferior ORR ($P = .027$) and PFS ($P = .022$) in m-cohort compared to wt-cohort. **Conclusion:** IO based treatments seem not detrimental for patients harboring novel driver alteration. Adding CT could improve modest responses to IO alone. Confirmation on larger datasets is required.

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide with an estimated 2 million of new cases and 1.76 million deaths per year.[1] Treatment options for patients with advanced non–small-cell lung cancer (aNSCLC) have been historically based on platinum-doublet chemotherapy. However, during the past decade, with the advent of molecular characterization and PD-L1 expression evaluation, target agents (TA) and immunotherapy single agent (IO) or in combination with platinum chemotherapy (CT-IO) have revolutionized the clinical practice scenario.[2] In aNSCLC patients harboring actionable oncogenic driver alterations, such as sensitizing *EGFR* mutations, *ALK, ROS1,* and *NTRK* rearrangements, and *BRAF* V600E mutation (BRAF V600E), TA have been approved in the first-line setting, with an overall response rate (ORR) of 60% to 80%.[2] Moreover, novel driver alterations such as *MET* exon 14 skipping mutations (METex14), *RET* rearrangements (RETr), *HER2* point mutations (HER2mut)/exon 20 insertions (HER2ex20), or uncommon *EGFR* mutations (uEGFR)/EGFR exon20 insertions (EGFRex20) have emerged as new therapeutic targets (Table 1 summarizes the abbreviations used for the novel driver alterations in this study).

The use of IO, such as anti-programmed cell death protein ligand 1 (anti-PD-L1), anti-programmed cell death protein 1 (anti-PD-1), and anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) monoclonal antibody (mAbs), with or without chemotherapy, has become the gold-standard treatment in non–oncogene-addicted aNSCLC. Nevertheless, its use among patients with sensitizing *EGFR* mutations, *ROS1* and *ALK* rearrangements is not recommended since it appears to show inferior efficacy.[3-6] Immunotherapy and CT-IO activity in patients with novel driver alterations remains poorly defined because a few of these patients have been included in prospective clinical trials. According to retrospective real-world data, compared to non–oncogene-addicted aNSCLC, the ORR seems to be similar in *BRAF*[7,8] and *c-MET*,[9,10] lower in *RET*,[11,12] while data are less consistent in HER2mut/HER2ex20 or in EGFRex20 altered aNSCLC.[13,14] The aim of this study was to investigate outcomes of IO based treatments in aNSCLC patients harboring novel driver alterations (METex14, BRAFV600E and *BRAF* atypical mutation (BRAFat), RETr, HER2mut/HER2ex20 or uEGFR/EGFRex20) in a real-world data scenario.

Table 1 List of Novel Driver Alterations' Abbreviations

BRAFat	BRAF Atypical Mutation
EGFRex20	EGFR exon20 insertions
HER2ex20	HER2 exon 20 insertions
HER2mut	HER2 point mutations
METex14	MET exon 14 skipping mutations
RETr	RET rearrangements
uEGFR	uncommon EGFR mutations

Materials and Methods

This retrospective study was conducted within the retrospective/prospective observational multicentre trial APOLLO 11 (INT 128-22, NCT05550961) at the Thoracic Oncology Unit of the "Fondazione IRCCS Istituto Nazionale Tumori" and at the Oncology Unit of the Niguarda Cancer Center of Milan, Italy. The study protocol was approved by the Internal Review Boards (IRB) and the Local Ethics Committees. Patients data were collected in accordance with the Declaration of Helsinki, Good Clinical Practice and local ethical rules. All patients have signed informed consent for the use of their clinical data for research purposes at some time of their medical history. Variables collected included: age, Eastern Cooperative Oncology Group performance status (ECOG PS), gender, ethnicity, smoking history, body mass index (BMI), histology, tumor burden and metastatic sites at the beginning of IO, IO treatment type, best response to IO, prior lines of treatment. Molecular pathology for gene mutations or rearrangement was determined in tissue by polymerase chain reaction (PCR) or next-generation sequencing (Oncomine Comprehensive Assay Plus, through Ion GeneStudio S5 Prime with IonTorrent technology -Thermo Fisher Scientific, Life Technologies) or fluorescence in situ hybridization for rearrangements when other techniques were not available or not evaluable. PD-L1 expression was determined by immunohistochemistry using the Dako PD-L1 22C3 pharmDx assay (Dako, Glostrup, Denmark). Molecular analyses were performed on archival tissue and pathogenic alterations were defined according to the Catalogue of Somatic Mutations in Cancer (COSMIC) and ClinVar databases.

Study Population

Data from 528 consecutive aNSCLC patients from January 2016 to September 2022 were retrospectively collected. The following inclusion criteria were required: (1) cytological or histological diagnosis of aNSCLC (stage IV or IIIB/C not candidate for locoregional therapies with curative intent); (2) at least 1 cycle of IO or combination CT-IO in any line; (3) ECOG PS 0 to 2; (4) evaluation for novel driver alterations: METex14, BRAFV600E and BRAFat, RETr, HER2mut/HER2ex20 or uEGFR/EGFRex20. The included treatments were IO monotherapy with nivolumab, pembrolizumab, atezolizumab and CT-IO with carboplatin or cisplatin in combination with pembrolizumab and pemetrexed or paclitaxel. Patients were divided in 2 cohorts according to the molecular status: mutated cohort (m-cohort) with detected novel driver alterations and wild type cohort (wt-cohort) that included patients without common (*EGFR* sensitizing mutation, *ALK* translocation, *ROS1* translocation) or novel driver alterations.

Objective of the Study

Primary endpoint of the study was the assessment of progression free survival (PFS) and statistical differences in terms of PFS between m-cohort and wt-cohort. Secondary endpoints were differences in terms of OS, ORR and disease control rate (DCR) between the 2 groups and differences in terms of PFS, OS, ORR, and DCR between single mutated subgroups within m-cohorts (namely: METex14, BRAFV600E, BRAFat, RETr, HER2mut, HER2ex20, uEGFR, EGFRex20). Exploratory assessment included TA timing impact on IO outcomes within m-cohort and assessment of first line IO single agent's outcome differences between m-cohort and wt-cohort in the PD-L1≥50% subgroup. OS was defined as the time between IO treatment start and death from any cause/last follow-up, while PFS was defined as the time between the IO treatment start and disease progression or death from any cause. Radiological assessments consisted of total body computed tomography (TB CT) scan performed at baseline and thereafter in a variable time interval according to local clinical practice, approximately every 3 months, or whenever progressive disease (PD) was clinically suspected. Tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1, defined as complete (CR), partial response (PR), stable disease (SD) and PD. ORR was defined as the sum of CR and PR while DCR as the sum of CR, PR, and SD.

Statistical Analysis

Descriptive statistics were used for the analyzed variables. Differences in their distribution were compared through Fisher's exact test or chi-square test, as appropriate. Survivals were estimated by the Kaplan-Meier method, and the log-rank test was used to assess differences among subgroups, reporting hazard ratio (HR) value. A statistical significance was assessed by setting P value at 5%. The median follow-up (m-follow up) was estimated using the inverse Kaplan-Meier method. For the propensity score analysis, propensity scores were calculated by logistic regression and propensity score matching (PSM) was performed with a 1:1 nearest-neighbor matching method without replacement with a caliper control of 0.3. All statistical analyses were performed by R software (version 3.6.2 and version 4.3.1).

Results

Patients' Characteristics

Patient's characteristics are detailed in Table 2. In the m-cohort were included 84 patients. Among them, 5 patients showed BRAFV600E, 9 BRAFat, 26 METex14, 9 uEGFR, 13 EGFRex20, 7 HER2m, 7 HER2ex20, and 8 patients had RETr (Supplemental—Table S1 summarizes the BRAFat and uEGFR mutations). The wt-cohort included 444 patients. Median age was 70 years old (range 42-92) for the m-cohort and 67 (range 27-89) for the wt-cohort. Nonsquamous histology was the most frequent in both cohorts (93% and 82% for m-cohort and wt-cohort respectively), while in the m-cohort a higher number of females and never-smoker patients was reported (48% vs. 37% and 44% vs. 11%). As regards immunotherapy treatment type, most patients received IO single agent in both cohorts (67% and 80% in the m-cohort and wt-cohort, respectively), the remaining received CT-IO. The majority of patients received IO as first line treatment in both cohorts (65% and 49% in m-cohort and wt-cohort, respectively).

Within the m-cohort, 55% of patients received a TA during their clinical history (13 patients before and 33 patients after IO based treatment). A detailed list of m-cohort subgroups characteristics is shown in Table 3.

Activity and Efficacy Analyses in the Overall Population and in m-Cohort Subgroups

With a m-follow up of 37.9 months, mPFS was 4.8 months (0.95 CI, 4.03-5.83) and mOS was 25.1 months (0.95 CI, 19.5-37.4) in the overall population. No differences in mPFS (HR 1.02, P = .846: 5.53 months, 0.95 CI, 4.73-6.97 vs. 4.57 months, 0.95 CI, 3.87-5.83) while higher mOS (HR 0.52, P < .0001: 25.1 months, 0.95 CI, 19.5-37.4 vs. 9.37 months, 0.95 CI, 8.37-10.8) were found in the m-cohort compared to the wt-cohort. In the overall sample, 83/84 of m-cohort and 443/444 of wt-cohort were evaluable for best response. No differences were found between for m-cohort and wt-cohort for ORR (22.9% vs. 27.8%, P = .481) and DCR (57.8% vs. 51.2%, P = .543), respectively.

An exploratory evaluation between single subgroups within the m-cohort was done: BRAFat reported better mPFS compared to other groups (HR 0.32, P = .0162), whereas the EGFRex20 mutated group reported worst mPFS (HR 2.02, P = .0347).No differences were found in terms of OS between the different mutation's subgroups. Patients in uEGFR group showed worse DCR compared to other subgroups (P = .0303), whereas BRAFat patients had better ORR (P = .0262) (Table 4). IO outcome's differences within m-cohort are shown in Supplemental—Tables S2-4.

Activity and Efficacy Analyses in m-Cohort Subgroup Treated in First and Second Line

Among the overall sample, 77 evaluable patients for the m-cohort and 359 patients for the wt-cohort were treated in the first and second line setting. No differences in mPFS (HR 0.98, P = .905: 6.10 months, 0.95 CI, 5.10-8.5 vs. 4.67 months, 0.95 CI, 3.93-6.0) but increased mOS (HR 0.48, P = <.001: 26.17 months, 0.95

Table 2 Overall Patient's Characteristics

	m-Cohort	wt-Cohort	P[a]
Patients – n (%)	84 (16)	444 (84)	
Age—m (range)	70 (42-92)	67 (27-89)	
Gender—n (%)			
M	44 (52)	281 (63)	.07805
F	40 (48)	163 (37)	
Smoking status—n (%)			
Current/former smoker	47 (56)	395 (89)	<.0001
Never Smoker	37 (44)	49 (11)	
ECOG PS—n (%)			
0	32 (38)	143 (32)	.03342
1	48 (57)	233 (53)	
2	4 (5)	68 (15)	
Histology—n(%)			
Squamous	6 (7)	82 (18)	.01664
Nonsquamous	78 (93)	362 (82)	
PD-L1 expression – n (%)			
<1%	25 (30)	120 (27)	.3467
1%-49%	28 (33)	156 (35)	
≥50%	28 (33)	102 (23)	
NA	3 (4)	66 (15)	
N of metastatic sites—n (%)			
<3	18 (21)	208 (47)	<.0001
≥3	64 (76)	234 (53)	
NA	2 (2)	2 (0)	
Treatment type—n (%)			
IO	56 (67)	357 (80)	.007969
CT-IO	28 (33)	87 (20)	
Treatment line—n (%)			
First	55 (65)	218 (49)	<.0001
Second	23 (27)	141 (32)	
Further lines	6 (8)	85 (19)	

[a] Fisher exact test/chi-square test, as appropriate.

CI, 22.80-37.4 vs. 9.37 months, 0.95 CI, 8.33-11.0) were found in m-cohort compared to wt-cohort, respectively. We next compared single m-cohort's subgroups with wt-cohort. As shown in Table 5, no differences were identified in terms of mPFS between each m-cohort and wt-cohort. A benefit in terms of mOS was confirmed only for patients harboring METex14 (HR 0.54, $P = .031$: mOS 23.27, 95% CI, 13.53-NA).

Similar ORR (24.7% vs. 29.0%, $P = .5351$) and DCR (62.3% vs. 53.5%, $P = .1684$) were reported, for m-cohort and wt-cohort respectively (Table 5).

Analyses According to TA Timing Within the m-Cohort

To evaluate the role of TA timing on IO based treatment's activity and efficacy, we next compared outcomes between patients who received TA before vs. after IO. No differences were reported in terms of mPFS (HR 0.77, $P = .494$: 5.37 months, 0.95 CI, 2.07-NA vs. 3.45 months, 0.95 CI, 2.20-5.53, in patients receiving TA before or after IO respectively) and mOS (HR 0.55, $P = .24$: NA, 0.95 CI, 4.17-NA vs. 23.7 months, 0.95 CI, 19.47-NA). Both ORR and DCR were comparable in patients who received TA before or after IO (ORR 18.2 vs. 13.9 months, $P = .6593$; DCR 45.4% vs. 44.4%, $P = .5134$).

Analyses According to Immunotherapy Treatment Type Within the m-Cohort

Comparing IO monotherapy with CT-IO in m-cohort, there were no differences in terms of mPFS (HR 1.50, $P = .127$: 4.93 months, 0.95 CI, 2.63-6.70 vs. 6.9 months, 0.95 CI, 5.47-13.9) and mOS (HR 1.19, $P = .631$: 23.7 months, 0.95 CI, 17.7-NA vs. 25.1 months, 0.95 CI, 13.2-NA) for IO vs. CT-IO treatment groups, respectively. An advantage in terms of ORR and DCR for patients receiving CT-IO was found (ORR 14.3% vs. 40.7%, $P = .0160$ and DCR 48.2% vs. 77.8%, $P = .0152$).

Analyses in the PD-L1 ≥50% Population

Among patients with PD-L1≥50% treated with IO single agent in first line, inferior results were reported in terms of mPFS for m-cohort (HR 1.80, $P = .0432$: 4.73,0.95 CI, 1.97-17.7 vs. 7.60

Table 3 m-Cohort Patient's Characteristics

	METex14	BRAFV600E	BRAFat	uEGFR	EGFRex20	HER2mut	HER2ex20	RETr
Patients—n(%)	26 (30.9)	5 (5.9)	9 (10.7)	9 (10.7)	13 (15.5)	7 (8.3)	7 (8.3)	8 (9.5)
Age—median(range)	73 (42-92)	73 (54-82)	71 (53-82)	65 (45-83)	70 (44-78)	71 (46-79)	64 (44-71)	53 (43-77)
Gender								
M	18 (69.2)	3 (60)	7 (77.8)	6 (66.7)	5 (38.5)	3 (42.9)	2 (28.6)	0 (0)
F	6 (23.1)	2 (40)	2 (22.2)	3 (33.3)	8 (61.5)	4 (57.1)	5 (71.4)	8 (100)
Smoking status								
Current/former smoker	13 (50)	2 (40)	9 (100)	6 (66.7)	5 (38.5)	4 (57.1)	5 (71.4)	3 (37.5)
Never Smoker	13 (50)	3 (60)	0 (0)	3 (33.3)	8 (61.5)	3 (42.9)	2 (28.6)	5 (62.5)
ECOG PS								
0	6 (23.1)	2 (40)	4 (44.4)	4 (44.4)	5 (38.5)	2 (28.6)	4 (57.1)	5 (62.5)
1	20 (76.9)	2 (40)	4 (44.4)	4 (44.4)	7 (53.8)	5 (71.4)	3 (42.9)	3 (37.5)
2	0 (0)	1 (20)	1 (11.1)	1 (11.1)	1 (7.7)	0 (0)	0 (0)	0 (0)
Histology								
Squamous	4 (15.4)	0 (0)	0 (0)	1 (11.1)	1 (7.7)	0 (0)	0 (0)	0 (0)
Nonsquamous	22 (84.6)	5 (100)	9 (100)	8 (88.9)	12 (92.3)	7 (100)	7 (100)	8 (100)
PD-L1 expression								
<1%	4 (15.4)	0 (0)	4 (44.4)	4 (44.4)	6 (46.1)	4 (57.1)	2 (28.6)	1 (12.5)
1-49%	7 (26.9)	3 (60)	2 (22.2)	4 (44.4)	3 (23.1)	3 (42.9)	4 (57.1)	2 (25)
≥ 50%	14 (53.8)	1 (20)	3 (33.3)	1 (11.1)	4 (30.8)	0 (0)	1 (14.3)	4 (50)
NA	1 (3.8)	1 (20)	0 (0)					1 (12.5)
N of metastatic sites								
<3	4 (15.4)	2 (40)	4 (44.4)	4 (44.4)	3 (23.1)	1 (14.3)	1 (14.3)	1 (12.5)
≥3	22 (84.6)	3 (60)	5 (55.6)	5 (55.6)	10 (76.9)	6 (85.7)	6 (85.7)	7 (87.5)
Brain metastases - n (%)	5 (19.2)	0 (0)	3 (33.3)	2 (22.2)	2 (15.4)	3 (42.9)	2 (28.6)	2 (25)
Liver metastases -n(%)	4 (15.4)	0 (0)	0 (0)	1 (11.1)	1 (7.7)	1 (14.3)	0 (0)	0 (0)
Bone metastases -n(%)	16 (61.5)	0 (0)	3 (33.3)	4 (44.4)	3 (23.1)	4 (57.1)	3 (42.9)	2 (25)
Treatment type								
IO	19 (73.1)	5 (100)	6 (66.7)	8 (88.9)	4 (30.8)	4 (57.1)	1 (14.3)	4 (50)
CT-IO	7 (26.9)	0 (0)	3 (33.3)	1 (11.1)	9 (69.2)	3 (42.9)	6 (85.7)	4 (50)
IO treatment line								
First	18 (69.2)	3 (60)	9 (100)	5 (55.6)	5 (38.5)	4 (57.1)	5 (71.4)	6 (75)
Second	8 (30.8)	1 (20)	0 (0)	2 (22.2)	5 (38.5)	3 (42.9)	2 (28.6)	2 (25)
Further lines	0 (0)	1 (20)	0 (0)	2 (22.2)	3 (23.0)	0 (0)	0 (0)	0 (0)
TKI therapy								
Pre IO	3 (11.5)	1 (20)	0 (0)	2 (22.2)	5 (38.5)	0 (0)	1 (14.3)	1 (12.5)
Post IO	14 (53.8)	1 (20)	0 (0)	1 (11.1)	5 (38.5)	3 (42.9)	4 (57.1)	5 (62.5)
No TKI	9 (34.6)	3 (60)	9 (100)	6 (66.7)	3 (23.0)	4 (57.1)	2 (28.6)	2 (25)

months,0.95 CI 4.13-16.4),) (Figure 1). No difference in terms of DCR between m- and wt-cohort (52.6% vs. 59.5%, $P = .7753$), but higher ORR for wt-cohort (15.8% vs. 39.2%, $P = .06369$) were found.

PSM Analysis

Propensity scores were calculated for m- and wt-cohort using the following covariates: age, gender, smoking status, ECOG PS, histology, PD-L1 expression, number of metastatic sites, treatment type and treatment line. After matching, among the 134 patients selected (67 for the m-cohort and 67 for the wt-cohort), the standardized mean difference (SMD) of variables selected for PSM was low (range, −0.0057 to 0.1793) suggesting significant reduction of bias between the 2 groups (Supplemental—Table S5). In the matched sample, results of the previous analyses on the overall population were confirmed: no differences in mPFS (HR 0.96, $P = .853$: 5.53 months, 0.95 CI, 5.10-9.90 vs. 4.57 months, 0.95 CI, 2.93-8.33) and higher mOS (HR 0.58, $P = .0164$: 23.7 months, 0.95 CI, 18.9-31.7 vs. 10.6 months, 0.95 CI, 8.4-14.9) were found for the m-cohort compared to the wt-cohort. Also, no differences were found between m-cohort and wt-cohort in terms of ORR (25.8% vs. 32.8%, $P = .4802$) and DCR (61.2% vs. 55.2%, $P = .5993$), respectively.

Discussion

Although TA have demonstrated to be the gold standard treatment in aNSCLC patients harboring actionable oncogenic driver alterations, the use of other innovative agents, such as IO based

Table 4 m-Cohort Immunotherapy Outcomes and Intra m-Cohort Comparison

	METex14	BRAFV600E	BRAFat	uEGFR	EGFRex20	HER2mut	HER2ex20	RETr
Patients -n(%)	26 (30.9)[a]	5 (5.9)	9 (10.7)	9 (10.7)	13 (15.5)	7 (8.3)	7 (8.3)	8 (9.5)
Best response -n(%)								
CR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PR	5 (19.2)	0 (0)	5 (55.5)	1 (11.1)	1 (7.7)	3 (42.8)	3 (42.8)	1 (12.5)
SD	11 (42.3)	2 (40)	2 (22.2)	1 (11.1)	5 (38.5)	2 (28.6)	2 (28.6)	4 (50)
PD	9 (24.6)	3 (60)	2 (22.2)	7 (77.8)	7 (53.8)	2 (28.6)	2 (28.6)	3 (37.5)
ORR -n(%)	5 (19.2), *P* .899	0 (0), *P* .4791	5 (55.5), *P* .02624	1 (11.1), *P* .6768	1 (7.7), *P* .2803	3 (42.8), *P* .1933	3 (42.8), *P* .1933	1 (12.5), *P* .675
DCR -n(%)	16 (61.5), *P* .5233	2 (40), *P* .6449	5 (55.5), *P* .2933	2 (22.2), *P* .03039	6 (46.1), *P* .5074	5 (71.4), *P* .6938	5 (71.4), *P* .6938	5 (62.5), *P* 1
OS -m(0.95CI)	23.3 (13.5-NA), *P* 0.831, HR 1.07	NA (4.43-NA), *P* .567, HR 0.66	25.1 (14.8-NA), *P* .959, HR 1.03	26.2 (4.43-NA), *P* .55, HR 1.30	17.7 (7.17-NA), *P* .518, HR 1.31	31.7 (18.9-NA), *P* .652, HR 0.76	18.0 (11.0-NA), *P* .495, HR 1.44	37.4 (27.1-NA), *P* .231, HR 0.49
PFS -m(0.05CI)	5.40 (2.6-13.87), *P* 0.864, HR 1.04	3.33 (2.63-NA), *P* .209, HR 1.81	17.0 (9.90-NA), *P* .0162*, HR 0.32	2.1 (1.8-NA), *P* .931, HR 1.03	5.17 (2.73-NA), *P* .0347[a], HR 2.02	9.70 (6.27-NA), *P* .669, HR 0.84	4.20 (2.80-NA), *P* .97, HR 0.98	5.1 (1.87-NA), *P* .597, HR 1.25

[a] 1 NA for best response.

Table 5 First- and Second-Line Setting Immunotherapy Outcomes for m-Cohort Subgroups Compared to w-Cohort

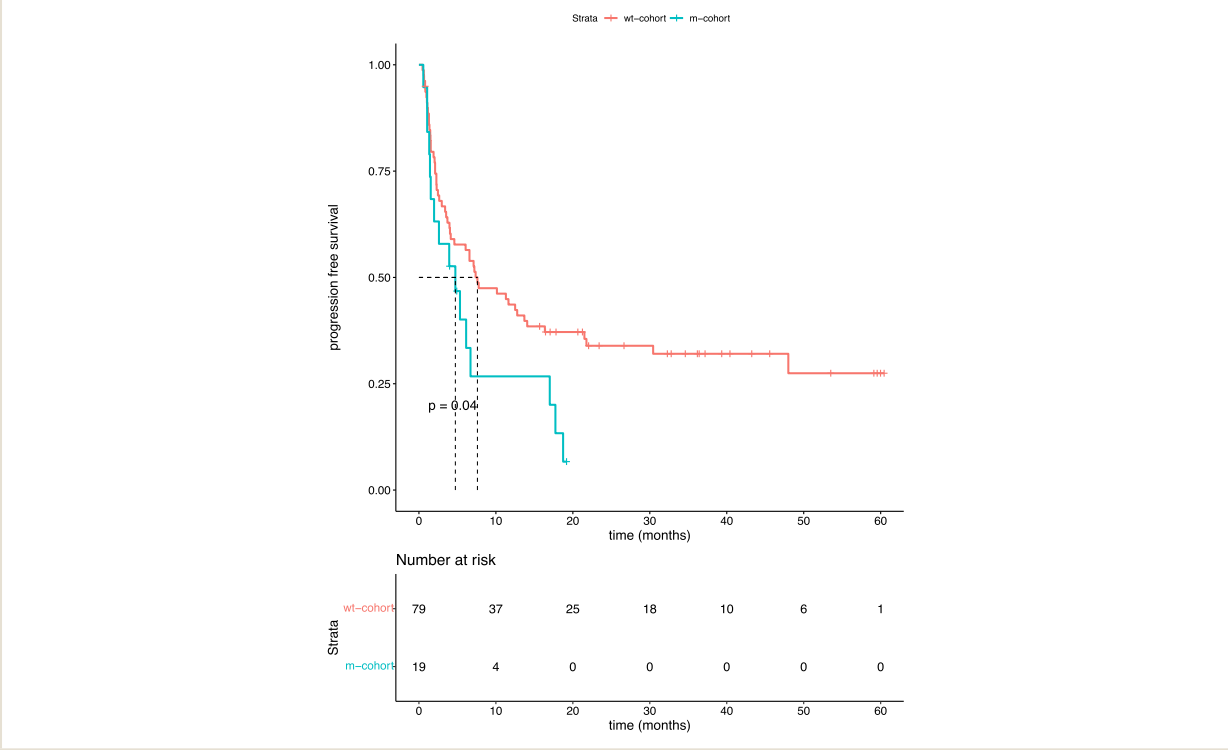
	n	ORR	DCR	PFS	OS
Wild type	359	29.0	53.5	4.67 (95% CI, 3.93-6)	9.37 (95% CI, 8.33-11)
m-cohort	77	24.7 (*P* .5351)	62.3 (*P* .1684)	6.10 (95% CI, 5.10-8.50), *P* .905, HR 0.98	26.17 (95% CI, 22.80-37.4), *P* <.001, HR 0.48
BRAFV600E	4	0 (*P* .5817)	50 (*P* 1)	4.67 (95% CI, 1.60-NA), *P* .349, HR 1.60	NA (95% CI, 2.63-NA), *P* .122, HR 0.21
BRAFat	9	55.6 (*P* .1322)	77.8 (*P* .1875)	17.00 (95% CI, 9.90-NA), *P* .067, HR 0.44	25.07(95% CI, 14.83-NA), *P* .173, HR 0.50
RETr	8	12.5 (*P* .4481)	62.5 (*P* .7296)	5.10 (95% CI, 1.87-NA) *P* .69, HR 1.18	37.43 (95% CI, 27.13-NA) *P* .051, HR 0.32
uEGFR	7	14.3 (*P* .6782)	28.6 (*P* .2606)	2.87 (95% CI, 1.80-NA) *P* .872, HR 0.94	26.73 (95% CI, 13.5-NA) *P* .147, HR 0.48
EGFRex20	10	10.0 (*P* .2931)	60.0 (*P* .7573)	5.05 (95% CI, 1.53-NA) *P* .0969, HR 1.71	17.67 (95% CI, 13.90-NA) *P* .245
HER2ex20	7	42.9 (*P* .4216)	71.4 (*P* .4583)	4.20 (95% CI, 2.80-NA) *P* .913, HR 1.04	18.02 (95% CI, 10.97-NA) *P* .276, HR 0.57
HER2mut	7	42.9 (*P* .4216)	71.4 (*P* .4583)	9.70 (95% CI, 6.27-NA) *P* .793, HR 0.90	31.07 (95% CI, 18.87-NA) *P* .104, HR 0.38
METex14	26	20.0 (*P* .4639)	65.4 (*P* .3307)	5.40 (95% CI, 2.60-13.9) *P* .753, HR 1.07	23.27 (95% CI, 13.53-NA) *P* .031, HR 0.54

treatment, is still controversial. Harboring common driver alterations, such as common sensitizing *EGFR* mutations or *ALK* rearrangements, has been largely used as an exclusion criterion in IO or CT-IO randomized controlled trials (RCTs). Moreover, most data, derived from RCTs, have provided information just for common EGFR mutation and *ALK* translocation while the efficacy of IO based therapies in NSCLC patients with novel drivers is still unclear.[15-18] Response to IO-based treatment depends on a complex interplay between tumor cells and tumor microenvironment (TME), resulting in immunosuppression or immunosensitivity.[19,20] Both tumor genetic alterations and TA may impact in different ways on TME. Thus, the evaluation of IO therapies in each different novel driver alteration in NSCLC has become of particular interest. Previously, Mazieres et al. in the IMMUNOTARGET registry have analyzed efficacy and activity of IO monotherapy in aNSCLC patients with cEGFR, HER2ex20, *KRAS* mutation, *BRAF* (exon 15) mutation, *MET* amplification or METex14, *ALK,* and *ROS1* translocation or RETr. They concluded that, although some oncogene addicted tumors derived a certain benefit from IO treatment, this option must be considered only after the exhaustions of TA and CT. In fact, in non *KRAS* mutant NSCLC IO activity alone seems to be unsatisfying.[3] Moreover, also *KRAS* mutated NSCLC has different outcome according to type of mutations, and in particular G12D mutant tumor could show worse outcomes to PD-(L)1 blockade.[21]

To our knowledge, this is the first retrospective study evaluating the use of IO, alone or in combinations with CT, in NSCLC harboring novel driver alterations (METex14, BRAFV600E, BRAFat, RETr, HER2mut/HER2ex20, uEGFR/EGFRex20) in comparison to a wt-cohort in a real-world setting and considering the TA timing according to the IO based treatment.

In our case series, the efficacy and activity of IO based treatments were similar among m-cohort and wt-cohort, since no statistical differences were observed in terms of ORR, DCR and PFS, both

Figure 1 PFS in PD–L1≥50% treated with first line IO single agent.



in the overall population, in matched sample and by type of treatment received (Supplemental Material S6 and Table S7), indicating a maintained benefit regardless of clinical characteristics and the addition of CT to IO.

An advantage was shown for OS in the m-cohort, both in the overall populations and in the first-second line setting only. Possible explanations of this benefit may include a more favorable clinical profile of m-cohort (higher percentage of nonsmoker, ECOG PS0-1, nonsquamous histology, lower burden patients) or a potential impact of TA administration. However, first hypothesis was disproved since the OS advantage for m-cohort was confirmed at the matched analysis, after balancing the samples for clinical variables. Moreover, even the exposure to TA did not affect OS ($P = .588$). Interestingly, patients not exposed to TA revealed better DCR ($P = .008$) and PFS ($P < .001$) than patients who received it. These results may reflect a condition of heavily pretreatment and worse clinical status for patients pretreated with TA. Indeed, IO treatment is frequently used as last option in patients harboring an alteration for which a TA is available.

Intriguingly, among every single subgroup of alterations, an advantage in term of OS was also identified for METex14 compared to wt-cohort (HR 0.54, $P = .031$: mOS 23.27, 95% CI, 13.53-NA). Since in our case series METex14 was the most represented alteration, a better OS in the overall m-cohort may be driven by this subgroup of patients. MET/HGF axis activation has been correlated to immune-escape in several preclinical and clinical studies, resulting in either increased expression of PD-L1[22-24] or a modulation of TME. In fact, MET activation could induce M2 macrophages differentiation, inhibition of dendritic cells, down-regulation of T-cells killing activity or mobilization of neutrophils to tumor niche with a transition to an immunosuppressive TME.[25-27] On the other hand, MET inhibition can restore immunosensitive TME, thus providing a rationale to combined IO and anti-MET treatment.[27] Of note, in our case series, the majority of patients has received TA after IO treatment (overall 33 of 46, 72%; METex14 14 of 26, 54%), not allowing us to draw considerations inherent a possible impact of an upfront TA modulation on TME and consequently IO response. Of note, nonstatistically significant longer OS was shown in METex14 patients that have received TA after IO (19.47 vs. 9.37 months, HR 1.24, $P = .495$, Supplemental—Material S8), thus providing a possible increase in OS in the overall m-cohort due to subsequent therapies driven by this subgroup.

For further explore the role of IO based treatment within the mutated subgroups, when every single driver alteration was evaluated, BRAFat showed better response and better PFS both compared to the whole m-cohort and to EGFRex20 and BRAFV600E subgroup (DCR 55.5 vs. 46.1 and 40%, PFS 17 vs. 5.17 and 3.33 months, respectively) (Supplemental—Table S2-4). It has already been demonstrated in melanoma patients that BRAF mutated tumors are able to derive benefit both from targeted and IO treatment.[28] In NSCLC, better outcomes with IO in BRAF mutated tumors, particularly non-V600E, could be explained since higher

smoke exposure, PD-L1 expression and high tumor mutational burden (TMB) levels were observed.[7,29] Previously, Dudnik et al. (10 patients) and Guisier et al. (18 patients) have demonstrated a potential role of IO in BRAFat NSCLC (PFS 3.7 and 4.9 respectively).[7,30] However, recent research evaluating the use of IO in BRAFat and BRAFV600E NSCLC failed to show any significant difference in terms of response and survival among these 2 subgroups (DCR 71 vs. 50% and PFS 10.8 vs. 10.5 months respectively).[31] A preclinical study evaluating TME composition in BRAFat and BRAFV600E did not show relevant differences compared to wt aNSCLC, suggesting that these driver mutations do not affect immunomodulation and giving a possible explanation for similar IO-derived benefit in these patients.[32]

Furthermore, in our sample uEGFR NSCLC demonstrated worse DCR and EGFRex20 worse PFS in comparison with other targetable alterations taken together. Yamada et al observed higher response (HR 0.047; 95% CI, 0.004-0.557, $P = .015$) and PFS (256 vs. 50 days, HR 0.288; 95% CI, of 0.13-0.63; $P = .003$) during IO-based treatment in uEGFR mutant aNSCLC (7 patients, 3 uEGFR, and 4 EGFRex20) compared to common EGFR mutations, suggesting a different behavior of these 2 classes of alterations. However, no comparison between uEGFR and EGFRex20 was performed.[33] Lau et al. have shown that different *EGFR* mutations (5 patients uEGFR, 6 patients EGFRex20) may have a distinct response to IO (ORR EGFRexon20 50%, other EGFR 11%).[14] It has been quite established that aNSCLCs with common EGFR mutations show lower TMB and immunosuppressive TME compared with wt-patients, thus suggesting potential resistance to IO. On the contrary, less evidence is available regarding immunomodulation by uEGFR and EGFRex20 tumor cells. Recently, a Chinese group has evaluated TCGA data regarding immune microenvironment of *EGFR* mutant aNSCLC. Among 98 *EGFR* mutant patients, common EGFR tumors (53 patients) showed higher myeloid dendritic cells levels by microenvironment cell populations-counter compared to other mutations (12 patients), implying a less immunosuppressive TME in uEGFR patients.[34]

Lastly, even if the addition of CT to IO in our sample has demonstrated a better DCR and ORR in m-cohort, no difference in survival was reported. Recently, Chen and co-authors have demonstrated that the addition of IO to CT increased response and survival in 164 patients with NSCLC and *EGFR* common mutation compared to CT alone.[35] However, in a larger but heterogeneous cohort including different driver mutant NSCLCs (246 patients divided in: *EGFR* common 54.9%, *KRAS* 32.9%, ALK 5.3%, HER2mut 2.9%, *ROS1* 1.2%, *MET* 1.2%, *RET* 0.8%, and BRAF non-V600 0.8%; 170 patients treated with CT-IO vs. 76 with CT alone), adding IO has not shown a significant benefit.[36] The lack of differences in this study may rely on the small number of patients for each specific subgroup. Of note, in our sample, PD-L1>50% NSCLC patients treated in first line with IO showed inferior PFS and ORR in m-cohort. These findings are consistent with previous literature data in common EGFR NSCLC, suggesting that the expression of PD-L1 does not correlate to better response to IO also in novel driver altered NSCLCs.[37] Taken together, although conflicting, these data suggest that the addition of CT may have some impact, even if limited, in overcoming the small response observed with IO single agent treatment in this population, especially since PD-L1 is not a reliable marker for oncogene addicted NSCLCs.

This study has several limitations. First, the retrospective nature of the analysis and the small incidence of novel alterations in NSCLC affecting the sample size, limit the power of the results. Specifically, the limited number of patients for each subtype of alteration does not provide sufficient understanding of the differences according to the type of driver. As a result, the overall findings may be driven by the most represented subgroup (METex14), and could not reflect the entire mutated population. Other limitations related to the retrospective design of the study include the variability in technologies used to detect alterations, potentially affecting mutations' detection in the wt-cohort, and the lack of standardization in radiological evaluation timing. Moreover, the value of TMB was not available for our patients, not allowing us to correctly evaluate the impact of gene alterations on tumor immunogenicity. In addition, IO based treatment were heterogeneous both as drug and line. Finally, only some patients received a specific TA according to the presence of the alteration.

Conclusion

To conclude, although with some limitations, this study demonstrated that IO based treatments might not be detrimental for patients harboring novel driver alteration *per se* and adding CT may have a limited but positive impact in overcoming the modest responses observed with IO single-agent therapy. Further evaluation, both clinical and translational, of the potential role of IO in novel driver oncogene addicted NSCLCs should be conduct in larger cohorts of homogeneous population for single type of driver alteration. This would allow to assess the benefit from IO of each specific subtype and also to understand which ones need CT addition to derive a benefit from IO, overcome intrinsic lack of immunogenicity and optimize treatment results.

Clinical Practice Point

- Researchers have provided multiple evidence that EGFR and ALK mutated non–small-cell lung cancers derive poor benefit from immunotherapy (IO). For novel driver alterations (MET exon 14 skipping, BRAF V600E and atypical mutations, RET and NTRK rearrangements, EGFR/HER2 uncommon mutations, or exon 20 insertions) the potential role of IO based treatments is not so well clarified, although some initial data exist.
- This study suggests that, overall, NSCLC harboring novel driver alterations derive similar benefit from IO compared to wild-type NSCLC. However, it also suggests that there might be a different degree of advantage from IO for these alterations, with BRAF atypical mutations being the most responsive to IO and EGFR/HER2 uncommon mutations or exon 20 insertions having the worst outcomes. Also, a trend towards better results with the addition of chemotherapy to IO (CT-IO) is reported in the study.
- Taken all together, these data suggest that the use of IO in NSCLC harboring novel driver alterations should not be discouraged, but the single mutation type should be considered when deciding to administer IO based treatments to these patients. Also, it could

be considered to add a chemotherapy to IO in order to improve treatment outcomes for this special category.

Disclosure

Diego Signorelli declares personal fees from AstraZeneca, Merck Sharp and Dohme, Boehringer Ingelheim, BMS, Roche and Sanofy. Arsela Prelaj declares personal fees from AstraZeneca, Italfarmaco, F. Hoffmann-La Roche, BMS. Roberto Ferrara declares advisory role from Merck Sharp and Dohme. Claudia Proto declares personal fees from Italfarmaco, AstraZeneca, BMS and Merck Sharp and Dohme. Giuseppe Lo Russo provided consultation, attended advisory boards and/or provided lectures for the following organizations, from whom received honoraria or education grants: Merck Sharp and Dohme, Takeda, Amgen, Eli Lilly, B.M.S., F. Hoffmann-La Roche, Italfarmaco, Novartis, Sanofi, Pfizer, G.S.K. and AstraZeneca. Marina Chiara Garassino declares personal financial interests with the following organizations: AstraZeneca, Merck Sharp and Dohm, International GmbH, BMS, Boehringer Ingelheim Italia S.P.A, Celgene, Eli Lilly, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, F. Hoffmann-La Roche, Takeda, Seattle Genetics, Mirati, Daiichi Sankyo, Regeneron, Merck, Ose Immuno Therapeutics, Blueprint, Jansenn, Sanofi; she also declares Institutional financial interests with the following organizations: Eli Lilly, Merck Sharp and Dohm, Pfizer (MISP); AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.P.A, Celgene, Eli Lilly, Ignyta, Incyte, MedImmune, Novartis, Pfizer, Roche, Takeda, Tiziana, Foundation Medicine, Glaxo Smith Kline GSK, Spectrum pharmaceuticals. Paolo Marchetti provided consultation, attended advisory boards and/or provided lectures for the following organizations, from whom received honoraria or education grants: F. Hoffmann-La Roche, Merck Sharp and Dohme, BMS, AstraZeneca, Boehringer-Ingelheim, Eli Lilly, Novartis and Pfizer. Filippo de Braud provided consultation, attended advisory boards and/or provided lectures for the following organizations, from whom received honoraria or education grants: Amgen, AstraZeneca, Boehringer-Ingelheim, BMS, Eli Lilly, F. Hoffmann-La Roche, Ignyta, Merck Sharp and Dohme, Merck Serono, Novartis, Pfizer. Sara Manglaviti: advisory board for Italpharma; travel accommodation by MSD and Sanofi outside the submitted work. Mario Occhipinti: advosry board for BMS and personal fee by MSD. Laura Mazzeo: travel accomodation by Sanofi. Marta Brambilla: travel accomodation by Lilli and Leo Pharma, Teresa Beninato: travel accomodation by Lilli, MSD, BMS and Sanofi. The other authors report no conflict of interest.

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Appendix

Table S1, Table S2, Table S3,Table S4, Table S5, Table 6 Table S7, Table 8

Table S1 List of BRAF atypical and uncommon EGFR mutations

BRAF_At	n	Type
class 2	5	K601E
		L597V
		G469V
		G469V
		G469V
class 3	4	D594N
		D594N
		L584F
		N581S
u_EGFR	9	I890M
		R776H
		G588S
		G917R
		L861Q
		V834I
		A675G
		V292M
		S768I

Table S2 PFS comparison with IO/CT-IO within m-cohort

	BRAFat	BRAFV600E	EGFRex20	uEGFR	HER2ex20	HER2mut	METex14
BRAFV600E	**0.0343**						
EGFRex20	**0.0076**	0.8767					
uEGFR	0.3147	0.9109	0.8593				
HER2ex20	0.1881	0.6179	0.4574	0.8767			
HER2mut	0.1881	0.1508	**0.0343**	0.9590	0.9313		
METex14	0.1881	0.7822	0.4601	0.9606	0.9590	0.8593	
RETr	0.1881	0.8593	0.7822	0.9313	0.8767	0.7112	0.9313

Table S3 ORR comparison with IO/CT-IO within m-cohort

	BRAFat	BRAFV600E	EGFRex20	uEGFR	HER2ex20	HER2mut	METex14
BRAFV600E	0.0859						
EGFRex20	**0.0231**	1					
uEGFR	0.1312	1	1				
HER2ex20	1	0.2045	0.1011	0.2615			
HER2mut	1	0.2045	0.1011	0.2615	1		
METex14	0.0847	0.5562	0.6426	1	0.3265	0.3265	
RETr	0.1312	1	1	1	0.2821	0.2821	1

Table S4 DCR comparison with IO/CT-IO within m-cohort

	BRAFat	BRAFV600E	EGFRex20	uEGFR	HER2ex20	HER2mut	METex14
BRAFV600E	0.2657						
EGFRex20	0.2031	1					
uEGFR	0.0567	0.5804	0.3802				
HER2ex20	1	0.5581	0.3742	0.1262			
HER2mut	1	0.5581	0.3742	0.1262	1		
METex14	0.6824	0.3644	0.4772	0.0523	1	1	
RETr	0.6199	0.5921	0.6594	0.1534	1	1	1

Table S5 Standardized mean difference comparison of baseline characteristics befor and after matching m- and wt-cohort.

Baseline characteristics	SMD before matching	SMD after matching
Age	0.0234	-0.0057
Gender (female vs male)	0.2096	0.1793
Smoking status (current/former vs never-smoker)	-0.6464	-0.0604
ECOG PS	-0.3156	0.1085
Histology (non squamous vs squamous)	0.3601	-0.0560
PD-L1 expression		
<1%	-0.0248	-0.0323
1-49%	-0.1333	-0.0314
>/= 50%	0.1574	0.0627
N of metastatic sites (<3 vs >/=3)	-0.5948	0.1063
Treatment type (IO vs CTIO)	-0.3027	-0.0314
Treament line	-0.3981	-0.0997

Material S6 Activity and efficacy analyses in the overall population according to immunotherapy treatment type.

Among the overall sample, 413 patients received IO monotherapy and 115 patients received CTIO. For each treatment type group, outcomes of m-cohort were compared with outcomes of wt-cohort. Results are summarized in supplementary Table S7.

Table S7 Activity and efficacy analyses for m-cohort compared to wt-cohort according to treatment type

	IO treatment group			CTIO treatment group		
	m-cohort	wt-cohort	p	m-cohort	wt-cohort	p
Patients -n(%)	56 (13.6)	357 (86.4)		28 (24.3)	87 (75.7)	
ORR -n(%)	8 (14.3)	85 (23.9)	0.1545	11 (40.7)	38 (43.7)	0.9626
DCR -n(%)	27 (47.4)	158 (44.3)	0.6955	22 (78.6)	69 (79.3)	1
OS -m(0.95CI)	23.70 (17.67-NA)	9.43 (8.37-11.3)	**0.00167* (HR 0.5568)**	25.1 (13.23-NA)	9.3 (7.27-12.9)	**0.0205* (HR 0.4504)**
PFS -m(0.05CI)	4.93 (2.63-6.70)	3.80 (3.23-4.63)	0.443 (HR 1.1232)	6.90 (5.47-13.87)	7.07 (5.83-8.87)	0.685 (HR 0.9023)

Material S8 Overall survival in METex14 patients treated with TA after IO and wt-cohort

Among METex14 patients [n=26, of which 17 (65.3%) received TA], we selected only patients treated with TA after having received IO (n=14, 53.8%) and compared this population with wt-cohort in terms of OS. A trend towards better OS, although not statistically significant, was identified for METex14 patients treated with TKI before IO group compared to wt-cohort (HR 1.24, p= 0.495: 19.47 mo, 0.95CI 7.83-NA vs 9.37 mo, 0.95CI 8.37-10.8)