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Word Count:

Pages = 34; Abstract = 250 words (max: 250 words); Article = 1, 838 words (max: 2,500 words); Figures = 1; Tables = 2; References = 38 (max: 40); Supplementary Figures = 1; Supplementary Tables = 1; Supplementary Material = 1.

Abstract

Background SHIVA01 randomized trial compared efficacy of matched molecularly targeted therapy outside their indications based on a prespecified treatment algorithm *versus* conventional chemotherapy in patients with metastatic solid tumours who had failed standard of care. No statistical difference was reported between the two groups in terms of progression-free survival (PFS), challenging treatment algorithm. ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) recently defined criteria to prioritize molecular alterations (MAs) to select anticancer drugs. We aimed to retrospectively evaluate the efficacy of matched molecularly targeted agents (MTAs) given in SHIVA01 according to ESCAT Tiers.

Patients and methods MAs used in SHIVA01 were retrospectively classified into ESCAT Tiers and PFS and overall survival (OS) were compared using log-rank tests.

Results Hundred and fifty-three patients were treated with matched MTAs in SHIVA01. MAs used to allocate MTAs were classified in Tiers II, IIIA, IIIB, and IVA according to ESCAT. Median PFS was 2.0 months in Tier II, 3.1 in Tier IIIA, 1.7 in Tier IIIB, and 3.2 in Tier IVA (p=0.13). Median OS in Tier IIIB was worse than in Tiers II, IIIA and IVA (6.3 months *versus* 11.7, 11.2 and 12.1, p=0.002).

Conclusions Most MAs used to allocate therapy in SHIVA01 were shown to improve outcome in other tumour types (Tier IIIA). Worst outcome was observed in patients treated based on another type of alteration than the one reported to improve outcome (Tier IIIB), highlighting the crucial impact of the type of the alterations beyond the gene and the signaling pathway.

Key words Molecularly targeted agents, ESMO Scale for Clinical Actionability of molecular Targets (ESCAT), actionable molecular alterations, treatment algorithms, precision medicine, SHIVA01

INTRODUCTION

Molecularly targeted agents (MTAs) given based on specific molecular alterations (MAs) were shown to be highly effective in several cancer types, such as vemurafenib in V600E *BRAF*-mutated melanoma patients or gefitinib in *EGFR*-mutated non-small cell lung cancer (NSCLC) patients [1, 2]. Several actionable MAs were identified across tumour types raising the question of comprehensive molecular profiling clinical utility to guide therapy. Precision medicine trials have been designed to assess the value of molecular profiling to allocate therapy in a histology-independent way [3–5].

SHIVA01 (NCT01771458) was the first randomized trial comparing matched MTA *versus* conventional therapy in patients with any kind of metastatic solid tumour refractory to standard of care [3]. SHIVA01 was negative for its primary endpoint with no statistical difference in progression-free survival (PFS) between the experimental and the control groups suggesting that SHIVA01 treatment algorithm was not able to improve patient outcomes. Improving treatment algorithms used in precision medicine trials represents a major challenge [6].

Several scales of actionability have been developed aiming at grading the levels of evidence associated with MAs [7–11], the latest one being the ESMO Scale of Actionability of molecular Targets (ESCAT) [12]. We aimed to retrospectively classify the MAs used to allocate MTAs in SHIVA01 according to ESCAT, and to evaluate MTAs efficacy accordingly.

PATIENTS AND METHODS

SHIVA01 was a proof-of-concept open-label randomized controlled phase 2 trial conducted in France [3]. MTAs used in the experimental group were drugs given outside their indications. Patients treated with MTAs following randomization or at cross-over in SHIVA01 were included in the study.

All MAs used to allocate MTAs in SHIVA01 were classified according to ESCAT by assessing the level of evidence in the literature (Supplementary Figure S1). To this end, for each MA, we first searched for clinical trials performed with the MTA in the same tumour type that would support ESCAT levels of evidence Tiers I or II. If no data supported Tiers I or II, we then searched for clinical trials performed with the same MTA and based on the same MA but in other tumour types that would support Tier III. If no data supported ESCAT Tier III, we searched for preclinical and *in silico* data that would support Tier IV. MAs were classified in Tier V if no clinically meaningful benefit was reported.

PFS and overall survival (OS) according to the ESCAT levels of actionability were compared using log-rank tests (GraphPad Prism7).

RESULTS

Patient population

Hundred and one out of the 153 included patients (66%) were females (Table 1). Median age was 57 years [range: 14-86]. MAs used to allocate therapy involved the PI3K/AKT/mTOR pathway in 77 patients (50%), the hormone receptor pathway in 56 patients (37%), and the Tyrosine Kinase Receptor (TKR)/RAF/MEK pathway in 20 patients (13%). Most frequent cancer types were breast (17%), ovarian (16%), and colorectal (12%) cancer.

Classification of MAs according to ESCAT

Extensive justification of MAs' classification according to ESCAT is available Supplementary Material and Table S1.

PI3K/AKT/mTOR *pathway*

Among 77 patients treated with everolimus, 28 patients (36%) had a *PIK3CA* activating hotspot mutation, 43 patients (56%) a *PTEN* inactivation, five patients (6%) an *AKT*

mutation/amplification, and one patient (1%) a *STK11* inactivating mutation associated with a loss of heterozygosity (Table 2).

PIK3CA mutations, *PTEN* inactivations, and *AKT1* mutations were shown to predict the efficacy of everolimus in HER2-positive breast cancer patients [13]. These alterations were therefore classified in Tier IIA according to ESCAT for breast cancer, and in Tier IIIA for the other cancer types. *AKT* amplifications were classified in Tier IIIB based on data reported with *AKT* mutations. *STK11* inactivations were classified in Tier IVA, since only preclinical data supported the use of everolimus for this MA [14].

TKR/RAF/MEK *pathway*

ERBB2 amplifications were identified in two patients with NSCLC and urothelial cancer in SHIVA01. Given the OS benefit obtained with the combination of lapatinib and trastuzumab in *ERBB2*-amplified breast cancer patients [15], and the lack of clinical evidence supporting the use of this combination in *ERBB2*-amplified NSCLC and urothelial cancer patients, *ERBB2* amplifications were classified in Tier IIIA in these cancer types. *ERBB2* mutations were classified in Tier IIIB for the two patients with neuroendocrine and colorectal cancers treated with this combination, based on the *ERBB2* amplifications data.

KIT mutations are present in most GIST patients, explaining the high efficacy of imatinib in this patient population [16]. *KIT* exon 11 mutations were shown to predict the efficacy of imatinib in GIST [17], and were therefore classified in Tier IIIA in NSCLC. *KIT* mutations were classified in Tier IIB for melanoma, given the efficacy reported with imatinib in melanoma patients with KIT mutations/amplifications in a single-arm phase II trial [18]. *KIT* exon 18 mutations in ovarian cancer and *KIT* exon 15 mutations in hepatocellular cancer were classified in Tier IIIB, assuming the same functional impact of these mutations then for *KIT* exon 11 mutations.

EGFR amplifications in oesophageal cancers were classified in Tier IIA, given the OS improvement in *EGFR*-amplified tumours in a retrospective analysis of a randomized trial with gefitinib [19]. Based on these results and given the lack of clinical evidence in head and neck squamous cell carcinoma (HNSCC) and squamous cervical cancer, *EGFR* amplifications were classified in Tier IIIA for these cancer types.

BRAF V600E mutation are classified in Tier IA for melanoma patients treated with vemurafenib [20]. Based on these results and given the limited efficacy of vemurafenib in *BRAF* V600E-mutated colorectal cancer patients [21, 22], *BRAF* V600E mutations were classified in Tier IIIA for colorectal cancer.

The predictive value of *PDGFR*, *RET*, *LCK*, and *FLT3* alterations of *PDGFR*, *RET*, *LCK*, and *FLT3* inhibitors' efficacy was only evaluated in preclinical models [14, 23–25]. These MAs were therefore classified in Tier IVA.

Hormone receptor pathway

Hormone therapy based on the expression of estrogen and/or progesterone receptors (ER/PR) is standard of care in breast cancer [26]. Since antitumor activity was only reported in ER/PR-positive ovarian cancer [27], these MAs were classified in Tier IIB for ovarian cancer, and in Tier IIIA in all other cancer types.

Androgen receptor (AR) are expressed in prostate cancer, explaining the high efficacy of abiraterone in this patient population [28]. Antitumor activity was reported in AR-positive breast cancer patients in a single-arm phase II trial [29]. AR expression was therefore classified in Tier IIB in breast cancer, and in Tier IIIA in all other cancer types.

Efficacy of MTAs given in SHIVA01 according to ESCAT levels of evidence

In total, out of the 153 patients treated with matched MTA in SHIVA01, 98 patients (64%) had a Tier IIIA MA, 38 patients (25%) a Tier II, seven patients (5%) a Tier IIIB, and 10

patients (7%) a Tier IVA. No MAs were classified in Tier I because of the SHIVA01 design, and none in Tiers IVB and V.

Median PFS was 2 months [range: 0.5-18.2] in Tier II, 3.1 months [range: 0.4-18.0] in Tier IIIA, 1.7 months [range: 0.3-3.7] in Tier IIIB, and 3.1 months [range: 1.2-8.9] in Tier IVA (p=0.13) (Figure 1a). OS was worse in Tier IIIB than in Tiers II, IIIA and IVA (median OS of 6.3 months [range: 2.3-11.1] *versus* 11.7 months [range: 2.5-37.4], 11.2 months [range: 2.1-29.9], and 12.1 months [4.4-20.3], p=0.002) (Figure 1b).

DISCUSSION

Our study is, to our knowledge, the first study to retrospectively classify MAs used in a precision medicine trial according to ESCAT, and to reassess survival according to levels of actionability. Since ESCAT relies on published preclinical and clinical scientific data, the distribution in the different Tiers will certainly evolve with time.

Most MAs used to allocate therapy in SHIVA01 were MAs shown to improve outcome in other tumour types (ESCAT Tier IIIA). Worst outcome was observed in patients who were treated based on another type of alteration (example: amplifications *versus* activating mutations in oncogenes) in a specific gene than the one reported to improve outcome (ESCAT Tier IIIB). The functional impact of unvalidated MAs within actionable genes may be assessed using *in vitro*/*vivo* analyses [30]. Patients with MAs in Tier IVA had a longer OS than patients with MAs in Tier IIIB. Although the numbers are small, this result suggests that *in silico* functional analyses may be more informative than extrapolating potential functional impact of MAs relying on a same gene or pathway.

We encountered several limitations while classifying SHIVA01 MAs according to ESCAT. First, we noticed that the literature interpretation was subject to inter-individual variability. In an attempt to limit this variability, the classification was validated by several experts. Second, some MTAs considered as reference treatment in some indications were not

assessed in randomized trials with a molecular selection based on the MA of interest, as was the case for *KIT* mutations and imatinib efficacy in GIST [31]. Other scales of actionability have overcome this limitation by classifying in Tier I FDA-approved drugs in a specific tumour type [7, 9, 10]. Third, there is no guidance in ESCAT on how to classify MAs associated with a lack of efficacy in specific tumour types. We classified *BRAF* V600E mutations in Tier IIIA for colorectal cancer based on melanoma data. However, given the limited efficacy of vemurafenib in colorectal cancer [21, 22], this MA may be rather classified in Tier V. Fourth, ESCAT doesn't take into account the impact of coexisting MAs that may confer resistance to therapy. As an example, PI3K inhibitors were shown to be effective in patients with tumours harbouring a *PIK3CA* mutation, while coexisting *PIK3CA* and *KRAS* mutations have been reported to predict limited efficacy of PI3K inhibitors [32].

Several parameters may impact the efficacy of MTAs beyond ESCAT level of actionability such MTA's affinity for a specific target. No recommendation exists on what should be the minimum affinity of a drug for a target to claim that a MA should be considered as a relevant target. The importance of MTA specificity is well illustrated by *PIK3CA* mutation and the use of alpelisib, an α-specific PI3K inhibitor, classified in Tier IA in breast cancer based on the SOLAR-1 trial [33, 34] while the same mutation in the same tumour type is classified in Tier IIA for everolimus [13]. Despite the preclinical potency of sorafenib to inhibit RAF kinases [35], sorafenib did not demonstrate any efficacy in frequently *RAF*-mutated melanoma [36]. The efficacy of a drug can also be impacted by comedications or food that may influence pharmacokinetics, as well as an inappropriate dose reductions [37, 38].

CONCLUSIONS

The majority of MAs used in SHIVA01 to allocate therapy had a low level of actionability according to ESCAT. This might in part explain the negative result of SHIVA01. Taking into

account other MAs in a specific gene than the one shown to improve outcome (Tier IIIB) was associated with worst outcome. This highlights the crucial importance of the type of alteration beyond the gene and/or the signaling pathway itself. Patients with MAs classified in Tier IVA had a better outcome than Tier IIIB, suggesting the value of *in vitro* and *in vivo* data for predicting MTA efficacy based on a specific MA.

Conflict of interest :

All authors of the manuscript declared no conflict of interest.

Funding sources:

This work was supported by the grant ANR-10-EQPX-03 from the Agence Nationale de le Recherche (Investissements d'avenir) and SiRIC (Site de Recherche Intégré contre le Cancer). High-throughput sequencing was performed by the NGS platform of the Institut Curie, supported by the grants ANR-10-EQPX-03 and ANR-10-INBS-09-08 from the Agence Nationale de le Recherche (investissements d'avenir) and by the Canceropôle Ile-de-France.

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Figure 1. Kaplan–Meier estimates of (a) progression-free survival (PFS), and (b) overall survival (OS) of patients treated with matched molecularly targeted therapy in SHIVA01 according to ESCAT Tiers

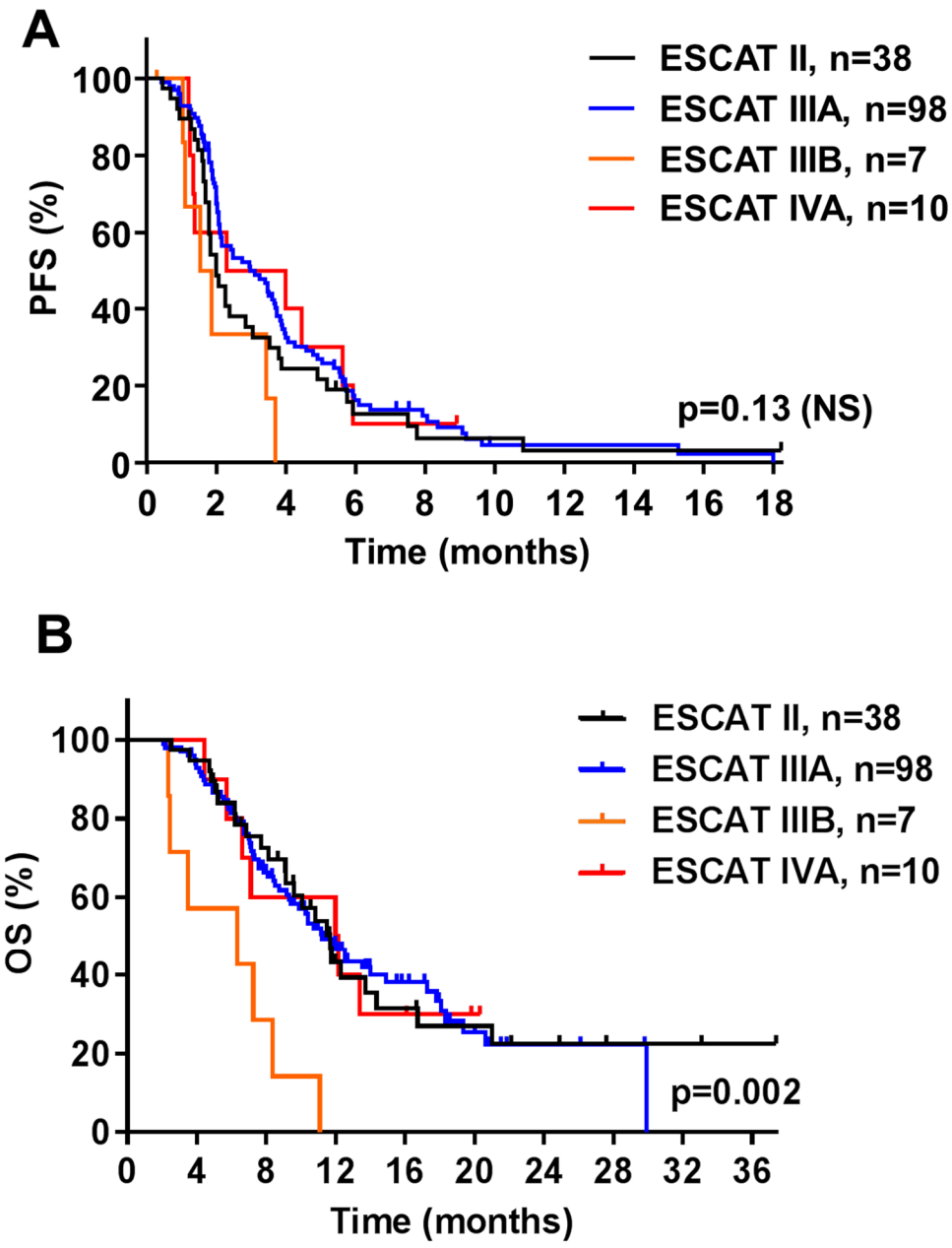

Table 1. Characteristics of patients treated with matched molecularly targeted therapy in SHIVA01

	N (%) **(Total = 153)**
Gender	
Male	52 (34)
Female	101 (66)
Tumour location	
PI3K/AKT/mTOR pathway	**77 (50)**
Colorectal	14 (9)
Breast	12 (8)
Ovarian	10 (7)
Lung	8 (5)
HNSCC	6 (4)
Endometrial	5 (3)
Cervical	5 (3)
Pancreatic	3 (2)
Sarcoma	2 (1)
Cholangiocarcinoma	2 (1)
Oesogastric	2 (1)
Anal	1 (1)
Melanoma	1 (1)
Adenoid cystic	1 (1)
Hepatocarcinoma	1 (1)
ACUP	1 (1)
Germline	1 (1)
Urothelial	1 (1)
Parotid	1 (1)
TKR/RAF/MEK pathway	**20 (13)**
Lung	3 (2)
Colorectal	3 (2)
Sarcoma	2 (1)
HNSCC	2 (1)
ACC	2 (1)
Neuroendocrine	2 (1)
Ovarian	1 (1)
Hepaticarcinoma	1 (1)
Melanoma	1 (1)
Oesogastric	1 (1)
Cervical	1 (1)
Urothelial	1 (1)
Hormone receptor pathway	**56 (37)**
Breast	14 (9)

Ovarian	14 (9)
Lung	4 (3)
Sarcoma	4 (3)
Cervical	3 (2)
HNSCC	2 (1)
ACUP	2 (1)
Urothelial	2 (1)
Cavum	2 (1)
Oesogastric	1 (1)
Colorectal	1 (1)
Kidney	1 (1)
Endometrial	1 (1)
Hepatocarcinoma	1 (1)
Mixopapillary ependymoma	1 (1)
Pancreatic	1 (1)
Parotid	1 (1)
Uveal melanoma	1 (1)

ACC = Adenoid Cystic Carcinoma; ACUP = Adenocarcinoma of Unknown Primary; HCC = Hepatocellular carcinoma; HNSCC = Head and Neck Squamous Cell Carcinoma

Table 2. Classification of molecular alterations used in SHIVA01 to allocate molecularly targeted agents according to ESCAT

Gene	Molecular alteration	Molecular targeted agent	Tumour type (N)	ESCAT Tier
PI3K/AKT/mTOR pathway				
PIK3CA	*PIK3CA* hotspot mutations	Everolimus	Breast (8)	IIA
			Colorectal (5)	IIIA
			Endometrial (3)	
			Cervical (2)	
			Ovarian (2)	
			Oesogastric (1)	
			Pancreatic (1)	
			Cholangiocarcinoma (1)	
			HNSCC (1)	
			ACC (1)	
			Anal (1)	
			Lung (1)	
			Cervical (1)	
PTEN	*PTEN* inactivation		Breast (4)	IIA
			Colorectal (9)	IIIA
			Ovarian (7)	
			Lung (5)	
			HNSCC (4)	
			Pancreatic (2)	
			Sarcoma (2)	
			ACUP (1)	

PTEN	*PTEN* inactivation	Everolimus	Cervical (1)	
			Parotid (1)	
			HCC (1)	
			Oesogastric (1)	
			Melanoma (1)	
			Germline (1)	
			Endometrial (1)	
			Cholangiocarcinoma (1)	
			Urothelial (1)	
AKT	*AKT1* amplification		HNSCC (1)	IIIB
			Lung (1)	
	AKT2 amplification		Ovarian (1)	
	AKT1 E17K mutation		Endometrial (1)	IIIA
			Cervical (1)	
STK11	D194L mutation + LOH		Lung (1)	IVA
TKR/RAF/MEK pathway				
ERBB2	Amplification	Lapatinib and trastuzumab	Lung (1)	IIIA
			Urothelial (1)	
	S792F mutation		Colorectal (1)	IIIB
	T862A mutation		Neuroendocrine Anal (1)	
KIT	D572G mutation (Exon 11)	Imatinib	Lung (1)	IIIA
	P838S mutation (Exon 18)		Melanoma (1)	IIB
	V852I mutation (Exon 18)		Ovarian (1)	IIIB
	M722V mutation (Exon 15)		HCC (1)	
EGFR	EGFR amplification	Erlotinib	HNSCC (1)	IIIA
			Cervical (1)	

BRAF	V600E mutation	Vemurafenib	Colorectal (1)	IIIA
PDGFRA/B	*PDGFRA* amplification	Sorafenib	ACC (2)	IVA
			Sarcoma (1)	
	PDGFRB amplification		Colorectal (1)	
	PDGFRA activation (intragenic deletion)		Sarcoma (1)	
	PDGFRA L655Y mutation		HNSCC (1)	
FLT3	M665T mutation	Sorafenib	Lung (1)	IVA
RET	Amplification	Imatinib	Oesogastric (1)	IVA
LCK	Amplification	Dasatinib	Neuroendocrine (1)	IVA
HR pathway				
ER-PR		Tamoxifen	Ovarian (11)	IIB
			Cervical (3)	IIIA
			Sarcoma (2)	
			Lung (2)	
			HNSCC (2)	
			Urothelial (1)	
			Cavum (1)	
			Colorectal (1)	
			Oesogastric (1)	
AR		Abiraterone	Breast (14)	IIB
			Ovarian (3)	IIIA
			Sarcoma (2)	
			Lung (2)	
			ACUP (2)	
			HCC (1)	

AR	Abiraterone	Endometrial (1)	IIIA
		Ependymoma (1)	
		Cavum (1)	
		Urothelial (1)	
		Kidney (1)	
		Pancreatic (1)	
		Parotid (1)	
		Uveal melanoma (1)	

ACC = Adenoid Cystic Carcinoma; ACUP = Adenocarcinoma of Unknown Primary; AR = Androgen Receptor; ER = Estrogen Receptor; HCC = Hepatocellular Carcinoma; HNSCC = Head and Neck Squamous Cell Carcinoma; LOH = Loss Of Heterozygosity; PR = Progesterone Receptor

PTEN inactivation = homozygous deletion of *PTEN,* or LOH associated with an inactivating mutation of *PTEN*, or LOH with loss of PTEN expression in immunohistochemistry, in all cases validated using immunohistochemistry

PDGFRA activation = intragenic deletion within PDGFRA validated by overexpression of PGDFRA using immunohistochemistry