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## **First-in-child phase I/II study of the dual mTORC1/2 inhibitor vistusertib (AZD2014) as monotherapy and in combination with topotecan-temozolomide in children with advanced malignancies: arms E and F of the AcSé-ESMART trial**

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### ► To cite this version:

Raphael J. Morscher, Caroline Brard, Pablo Berlanga, Lynley V. Marshall, Nicolas André, et al.. First-in-child phase I/II study of the dual mTORC1/2 inhibitor vistusertib (AZD2014) as monotherapy and in combination with topotecan-temozolomide in children with advanced malignancies: arms E and F of the AcSé-ESMART trial. *European Journal of Cancer*, 2021, 157, pp.268-277. 10.1016/j.ejca.2021.08.010 . hal-03487880

**HAL Id: hal-03487880**

**<https://hal.science/hal-03487880>**

Submitted on 16 Oct 2023

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**First in child phase I/II study of the dual mTORC1/2 inhibitor vistusertib (AZD2014) as monotherapy and in combination with topotecan-temozolomide in children with advanced malignancies: Arms E and F of the AcSé-ESMART trial**

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**Conflict of interest:** PM is employee of AstraZeneca, Cambridge, UK. The other authors have no conflict of interest declared.

**Key words:** paediatric relapsed refractory cancer, dual mTOR inhibitor, vistusertib, AZD2014, topotecan, temozolomide, molecular enriched phase I/II, PI3K/AKT/mTOR pathway.

### **Highlights**

- Dual mTOR inhibition by vistusertib is well tolerated in the paediatric population.
- Insufficient anti-tumour response to vistusertib monotherapy and with TOTEM.

- mTOR pathway activating mutations do not sensitize to vistusertib therapy.

**Running title:** Vistusertib in paediatric tumours

**Abstract**

**Aim:** Arms E and F of the AcSé-ESMART phase I/II platform trial aimed to define the recommended dose and preliminary activity of the dual mTORC1/2 inhibitor vistusertib as monotherapy and with topotecan-temozolomide in a molecularly enriched population of paediatric patients with relapsed/refractory malignancies. Additionally we evaluated genetic PI3K/AKT/mTOR pathway alterations across the MAPPYACTS trial (NCT02613962).

**Experimental design and Results:** Four patients were treated in Arm E and 10 in Arm F with a median age of 14.3 years. Main diagnoses were glioma and sarcoma. Dose escalation was performed according to the continuous reassessment method; expansion in an Ensign design. Vistusertib single agent administered at 75 mg/m<sup>2</sup> BID on 2 days/week, and vistusertib 30 mg/m<sup>2</sup> BID on 3 days/week combined with temozolomide 100 mg/m<sup>2</sup>/day and topotecan 0.50 mg/m<sup>2</sup>/day on the first 5 days of each 4-weekly cycle were safe. Treatment was well tolerated with the main toxicity being haematological. Pharmacokinetics indicates equivalent exposure in children compared to adults. Neither tumour response nor prolonged stabilization was observed, including in the 12 patients whose tumours exhibited PI3K/AKT/mTOR pathway alterations. Advanced profiling across relapsed/refractory paediatric cancers of the MAPPYACTS cohort shows genetic alterations associated with this pathway in 28.0% of patients, with 10.5% carrying mutations in the core pathway genes.

**Conclusions:** Vistusertib was well tolerated in paediatric patients. Study arms were terminated because of absence of tumour responses and insufficient target engagement of vistusertib observed in adult trials. Targeting the PI3K/AKT/mTOR pathway remains a therapeutic avenue to be explored in paediatric patients.

Clinical trial identifier: NCT2813135.

## Introduction

Despite current multimodal therapy approaches, children with relapsed/refractory tumours show poor response rates to salvage regimens and have dismal prognosis<sup>1</sup>. Advanced molecular characterization in these malignancies has revealed targetable alterations opening new opportunities for precision medicine approaches<sup>2-4</sup>. The rarity of paediatric cancers and the heterogeneity of molecular alterations mandate innovative efficient trial designs in order to satisfy the unmet medical need. Large academic and multi-stakeholder efforts are helping counteract the lack of access to novel therapies and facilitate standardized evaluation in the paediatric cancer population. In this context, AcSé-ESMART (European Proof-of-Concept Therapeutic Stratification Trial of Molecular Anomalies in Relapsed or Refractory Tumours; NCT02813135/EudraCT 2016-000133-40/ITCC-057) was developed as a proof-of-concept phase I/II platform trial, designed to explore the targeting of cancer cell survival pathways in a molecularly enriched paediatric patient population. Here we report the outcomes of two study arms targeting the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian (or mechanistic) target of rapamycin (mTOR) pathway. Cellular reprogramming by hyperactivation of the PI3K/AKT/mTOR pathway has been described across various paediatric and adolescent cancers<sup>5-7</sup>. The

serine/threonine kinase mTOR functions as a central node in integrating growth signals and nutrient sensing. It exerts its activity within two distinct complexes, mTORC1 and mTORC2<sup>8</sup>. Preclinical and clinical studies suggest that allosteric inhibition of mTOR by rapamycin analogues preferentially inhibits mTORC1 and is linked to persistent activity of mTORC2 due to suppression of negative feedback loops inducing AKT<sup>9,10</sup>. Direct inhibition of mTOR kinase affecting both signalling complexes has been shown preclinically to more effectively target cancer cell growth and survival linked to mTOR signalling<sup>11,12</sup>. Dual inhibition has also been demonstrated to destabilize the MYCN protein in neuroblastoma, inducing apoptosis and anti-tumour response<sup>13</sup>.

In this study we explored vistusertib (AZD2014), developed as a potent (low nanomolar) and selective inhibitor of the mTOR kinase<sup>14</sup> inhibiting downstream phosphorylation via both mTORC1 and mTORC2 through competitive targeting of active site adenosine triphosphate-binding. Based on promising preclinical activity<sup>15-17</sup> as well as clinical indicators and biomarker response in patient biopsies<sup>18</sup>, several clinical studies, including this study, were launched<sup>19-21</sup>.

The addition of mTOR inhibitors to chemotherapy such as topoisomerase I inhibitors and temozolomide has been shown to be effective<sup>22,23</sup>, reverse resistance<sup>24</sup> and display synergistic activity<sup>25</sup>. Similarly the clinical application of vistusertib was expected to be in combination with established chemotherapy. In the target patient tumour spectrum a topotecan-temozolomide (TOTEM) combination had shown activity in neuroblastoma<sup>26</sup> and medulloblastoma<sup>27</sup> with a favourable safety profile. Arms E and F of AcSé-ESMART therefore explored vistusertib treatment alone and in combination with TOTEM in a molecularly enriched patient population.

## **PATIENTS AND METHODS**

### **Study population and design**

Patients with relapsed or refractory malignancy who had advanced molecular profiling of their tumour were eligible. Main inclusion criteria: age < 18 years or paediatric malignancy, no standard curative treatment option, evaluable or measurable disease, performance status  $\geq$  70% and adequate organ function.

Arm E included patients who elected not to receive cytotoxic chemotherapy or for whom the TOTEM combination was not considered an appropriate choice. The goal was to achieve maximal enrichment with patients whose tumours` harboured activating core PI3K/AKT/mTOR pathway alterations, activating mutations in receptor tyrosine kinases, or *MYC/MYCN* amplification and/or increased expression. Arm F included patients for whom TOTEM was considered an appropriate treatment choice and whose tumours harboured any of the afore-mentioned alterations with an enrichment approach of at least 50%.

The primary objectives of the phase 1 parts of arms E and F were, respectively, to validate the adult single agent recommended phase II dose (RP2D) of vistusertib, and define the RP2D in combination with TOTEM. For the phase 2 parts, primary objectives were to determine the preliminary activity in patients enriched for specific molecular alterations.

The trial was approved by independent ethics committees, national medical authorities and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Informed consent/assent was obtained from all patients or/and legal representatives.

### **Treatment and dose levels**

In Arm E, vistusertib was given orally twice a day (BID) in an intermittent weekly schedule of two consecutive days on, followed by 5 days off in 28-day cycles. Patients on dose level 1 (DL1) received 75 mg/m<sup>2</sup> BID, equivalent to 100% of the adult RP2D<sup>18,21</sup>.

In Arm F, schedule and dose of vistusertib were chosen based on a combination with paclitaxel in adults where 50 mg BID was given in a weekly schedule of 3 consecutive days on, 4 days off, with the intention to maximize exposure during chemotherapy<sup>28</sup>. Temozolomide was given orally in a fasted state followed by topotecan intravenously on Days 1-5 of a 28-day cycle<sup>26,27</sup>. Starting DL1 was vistusertib 30 mg/m<sup>2</sup> BID, temozolomide 100 mg/m<sup>2</sup>/day and topotecan 0.5 mg/m<sup>2</sup>/day (66% paediatric TOTEM combination RP2D). In the absence of toxicity, TOTEM was to be escalated to the 100% paediatric combination RP2D, then vistusertib to 36 mg/m<sup>2</sup>. In case of toxicity, DL-1 used vistusertib at 20 mg/m<sup>2</sup>.

### **Statistical design**

In Arm E, DL1 was expected to be tested for dose validation in 2 cohorts of 6 + 4 patients. Arm F conducted dose escalation using the continuous reassessment method (CRM) targeting a dose associated with a 25% risk of DLT. The dose toxicity model was a one-parameter power model, where  $\alpha_1=0.12$ ;  $\alpha_2=0.25$ ,  $\alpha_3=0.40$  and  $\alpha_1=0.05$ . A Bayesian inference was used together with a weakly informative normal distribution prior. The dose escalation was completed when 10 patients had been treated at the dose recommended for expansion (RP2D). For both arms if one response or more was observed, expansion cohorts were to be accrued corresponding to an Ensign three-stage design ( $\alpha=\beta=10\%$ ,  $H_0=10\%$  and  $H_a=30\%$ ).

### **Study evaluations**

Adverse events (AE) assessed throughout the study were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03). Patients were considered evaluable for DLT after receiving more than 75% of treatment of the planned cycle 1 or had DLT<sup>29</sup>. Response assessment was performed every two cycles according to specific disease (RECIST, version 1.1<sup>30</sup>, RANO<sup>31</sup>).

### **Pharmacokinetics (PK)**

In patients treated in Arm E, limited sampling PK analysis for vistusertib was performed during cycle 1 on day 1 and day 9 (each pre-dose, at 0.5h, 1h, 2h, 4h, 8h), and trough levels on day 1 of cycles 2-4. For Arm F no PK was initially planned due to low theoretical risk of interaction. AZD2014 serum levels and PK using non-compartmental methods were determined following published protocols<sup>18</sup>.

### **Genetic alterations related to PI3K/AKT/mTOR pathway activation in paediatric relapsed/refractory tumours**

We leveraged the sequencing data of MoleculAr Profiling for Paediatric and Young Adult Cancer Treatment Stratification (MAPPYACTS; NCT02613962) to estimate the frequency of genetic alterations implicating activation of the PI3K/AKT/mTOR signalling axis or potential sensitivity to mTOR inhibition in a cohort of paediatric relapsed/refractory tumours. This cohort comprises contributory molecular profiles in 622 patients across the full spectrum of paediatric entities including 178 central nervous system (CNS) tumours, 245 sarcomas, 138 other solid tumours, 15 lymphomas and 46 leukaemias (Berlanga et al., in preparation). WES and RNA sequencing data had been analysed by a standardized pipeline<sup>3</sup> and variants prioritized by the interdisciplinary molecular tumour board (MTB) of bioinformaticians, biologists and clinicians.

## Results

### Patient characteristics

Fourteen patients were included and treated between November 2016 and June 2018, 4 in Arm E and 10 in Arm F. Patients' baseline characteristics are listed in Table 1. Eight patients had a CNS tumour, five had sarcomas. Three (Arm E) and nine patients (Arm F) were included whose tumours exhibited an alteration in the PI3K/AKT/mTOR pathway. All variants considered as potentially relevant by an interdisciplinary MTB are given in Figure 1 and as Supplementary Table 1. Half of the patients had received prior targeted treatment. For two patients treated with sonidegib or dasatinib, respectively, molecular profiling was performed after treatment. The other patients received treatment between profiling and study inclusion, namely pembrolizumab, bevacizumab (3 x), dasatinib and lenvatinib.

**Table 1: Patients' characteristics in Arm E and Arm F treated with vistusertib alone or topotecan-temozolomide (TOTEM).**

	Arm E Vistusertib N=4	Arm F Vistusertib + TOTEM N=10	Total N=14
<b>Age at study entry (years)</b>			
Median	15.2	13.7	14.3
[range]	[6.4-17.4]	[3.7-19.2]	[3.7-19.2]
<b>Age at Diagnosis (years)</b>			
Median	13.0	8.7	9.7
[range]	[5.7-14.8]	[2.2-17.8]	[2.6-17.8]
<b>Gender</b>			
Male	0 (0%)	5 (50%)	5 (36%)
Female	4 (100%)	5 (50%)	9 (64%)
<b>Lansky play scale / Karnofsky Score</b>			
100-90%	1 (25%)	6 (60%)	7 (50%)
80-70%	3 (75%)	4 (40%)	7 (50%)
<b>Histological Diagnosis</b>			
Rhabdomyosarcoma	-	3 (30%)	3 (21%)
Osteosarcoma	1 (25%)	1 (10%)	2 (14%)
Nephroblastoma	-	1 (10%)	1 (7%)
Medulloblastoma	-	2 (20%)	2 (14%)
Diffuse intrinsic pontine glioma	2 (50%)	-	2 (14%)

High grade glioma	-	2 (20%)	2 (14%)
Oligodendroglioma	-	1 (10%)	1 (7%)
Meningioma	1 (25%)	-	1 (7%)
<b>Initial diagnosis to inclusion (years)</b>			
Median [Min-Max]	2.2 [0.7-2.5]	2.7 [0.7-15.0]	2.2 [0.7-15.0]
<b>Disease status at study entry</b>			
Recurrent	1 (25%)	7 (70%)	8 (57%)
Refractory / progressive	3 (75%)	3 (30%)	6 (43%)
<b>Metastatic at study entry</b>			
Yes	1 (25%)	8 (80%)	9 (64%)
No	3 (75%)	2 (20%)	5 (36%)
<b>Site of metastatic disease</b>			
Liver	0	1 (10%)	1 (7%)
Lymph nodes	0	2 (20%)	2 (14%)
Lung	1 (25%)	3 (30%)	4 (29%)
Brain	0	5 (50%)	5 (36%)
Meningeal	0	1 (10%)	1 (7%)
<b>Prior treatment</b>			
Prior treatment lines median (range)	2 (1-3)	3.5 (2-7)	2.5 (1-7)
Chemotherapy	1 (25%)	10 (100%)	11 (79%)
High-dose chemotherapy	0 (0%)	2 (20%)	2 (14%)
Targeted systemic treatment	4 (100%)	3 (30%)	7 (50%)
Radiation therapy	4 (100%)	7 (70%)	11 (79%)
Surgical resection	2 (50%)	8 (80%)	10 (71%)

### Study treatment and dose-limiting toxicity

In Arm E, 4 patients were treated at DL1 with vistusertib 75 mg/m<sup>2</sup> BID. Two patients were not evaluable for DLT, one with meningioma because of a non-treatment-related AE and end of treatment at day 9, and one due to progression of the underlying diffuse intrinsic pontine glioma and inability to swallow tablets at day 4. None of the two evaluable patients experienced DLT.

In Arm F, 10 patients were treated with vistusertib and TOTEM at 2 dose levels. Two out of the first five patients treated at DL1 experienced dose-limiting prolonged thrombocytopenia Grade 3 and 4 necessitating platelet transfusions, the prior was associated with prolonged Grade 4 neutropenia. The following three patients were treated at DL-1 with vistusertib 20 mg/m<sup>2</sup> BID. One patient was not evaluable for DLT due to rapid tumour progression. The other patients did not experience DLT, so the CRM suggested to re-escalate; the subsequent 2 patients treated at DL1

experienced no DLT. The estimate of the risk of DLT at DL1 is 0.231 (95% Credibility interval =0.06, 0.46) which seems to qualify DL1 for the RP2D.

Overall, in 7 cycles of vistusertib monotherapy patients experienced 13 treatment-related AE, mainly Grade 1-2 general and gastrointestinal disturbances (Table 2). Sixteen cycles of vistusertib were given in combination with TOTEM. The 10 patients experienced 52 AEs considered treatment-related, mainly haematological and gastrointestinal. Main Grade 3 and 4 events were thrombocytopenia and neutropenia.

**Table 2: Treatment-related adverse events maximum grades**

Adverse Events per cycle	Arm E Vistusertib N=7 cycles in 4 patients			Arm F Vistusertib & TOTEM N=16 cycles in 10 patients		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
<b>Haematologic</b>						
Anaemia	-	1	-	5	2	-
Leukopenia	-	-	-	3	2	-
Lymphopenia	-	-	-	3	2	-
Neutropenia	-	-	-	-	3	3
Thrombocytopenia	-	-	-	-	7	1
<b>General</b>						
Headache	1	-	-	-	-	-
Fatigue	-	-	-	2	-	-
General muscle weakness	1	-	-	-	-	-
Anorexia	1	-	-	1	-	-
Fever	1	-	-	-	-	-
<b>Gastrointestinal</b>						
Nausea	2	-	-	4	-	-
Vomiting	1	-	-	2	-	-
Constipation	-	-	-	2	-	-
Abdominal pain	-	-	-	1	-	-
<b>Dermatologic</b>						
Mucositis oral	-	-	-	3	-	-
Dry skin	1	-	-	-	-	-
Redness lesions on hands	1	-	-	-	-	-
<b>Investigational/laboratory</b>						
Proteinuria	1	-	-	-	-	-
Hypophosphatemia	-	1	-	-	-	-
Hypothyroidism	1	-	-	-	-	-
ALAT / GGT / Bilirubin elevation	-	-	-	3	-	-
Hypercholesterolemia	-	-	-	1	-	-
Hypertriglyceridemia	-	-	-	1	-	-
Lipase increased	-	-	-	-	1	-

Data given as maximum AE per cycle for vistusertib as single agent and in combination with topotecan-temozolomide across dose levels according to NCI-CTC.

### **Antitumour activity**

In both arms 13 patients were evaluable for tumour response. Patient E-01 was not evaluable due to early treatment end for disease-related AE; the patient is still alive at study cut-off on March 31, 2021. One patient in Arm F (F-05) with high grade glioma experienced stable disease with -9% change in the target lesion after one cycle as best response but stopped treatment due to AE. All other patients were progressive at 1 or 2 cycles. Best treatment response with associated genetic alterations are given for each patient in Figure 1.

In arm E, the progression-free survival for individual patients was 3.3, 1.9, 1.1 and 0.2 months, respectively; the overall survival was 33.2 (alive), 9.6, 2.3 and 1.2 months. In Arm F, the median progression-free survival was 1.9 months [95% Confidence Interval: 0.8-2.3] and median overall survival was 4.8 months [95% Confidence Interval: 0.9-9.4].

### **Figure 1: Visualization of patient target lesion best response (top) and individual correlated genetic alterations.**

Clinically progressive disease without imaging was arbitrarily set to 100%. Rows indicate genes with respective names and columns indicate patients with Arm E / F and identifier on top. \* progressive disease due to new or increase of non-target lesions. Abbreviations: NA: not applicable; CP: clinically progressive disease; CNS: central nervous system.

### **Pharmacokinetic studies of vistusertib**

All four patients in Arm E treated at DL1 had PK samples at day 1 of Cycle 1 and two were also dosed at steady state on day 9. As shown in Figure 2 vistusertib was rapidly absorbed with mean time to peak of 2.0 hours. Terminal elimination half-life mean was 6.5 hours, mean exposure expressed as area under the curve (AUC) was 13000 h\*ng/mL and maximal concentration 3510 ng/mL. Details for individual patients and steady state are given in Supplemental Table 2.

### **Figure 2: Total plasma concentration and exposure of vistusertib**

A) Total plasma concentration of vistusertib monotherapy at 75 mg/m<sup>2</sup> BID in children on day 1 (N=4), given as mean ± standard deviation and B) day 9 steady state (N=2), given as mean ± range. Panel C) indicating area under the curve (AUC) exposure of vistusertib in single patients and comparison to adult AUC mean for vistusertib at 100 mg as reference. \* Adult AUC mean and range, data taken from Basu et al. 2015 <sup>18</sup>.

### **Alterations in PI3K/AKT/mTOR pathway in the MAPPYACTS pan-cancer cohort**

Out of the 622 patients in MAPPYACTS with contributory sequencing, single nucleotide and copy number alterations were reported in the core mTOR pathway in 65 cases (10.5%). Primary genes affected were *PTEN* (N=25), *PIK3CA* (N=14) and *AKT1* (N=9). Details are shown in Figure 3. The three tumour entities with the highest frequency of core mTOR pathway alterations were high grade glioma (26.2%), osteosarcoma (23.1%) and rhabdomyosarcoma (12.3%). We then expanded the analysis to additional alterations that could potentially provide sensitivity to mTOR targeting such as growth factor receptor alterations implicating downstream activating PI3K/AKT/mTOR pathway and *MYC/N/L*; additional 152 patients in this cohort

showed related mutations to a total of 34.9% of patients (Figure 3B, N=217 of 622). As variants in the mitogen activated protein (MAP) kinase pathway and TP53 alterations have been associated with resistance to mTOR inhibition<sup>32,33</sup> we investigated the co-occurrence with core mTOR pathway alterations. 16.9% of cases (N=11 of 65) showed also mutations in the RAS family or BRAF. Together with the apoptosis/transcription regulator TP53 (N=25 of 65) and cell cycle regulation (20 of 65), these pathways represent the three most frequently co-altered across this cohort. An overview of the mutation spectrum grouped by tumour type is given as Supplemental Figure 1.

**Figure 3: Core PI3K/AKT/mTOR-pathway and associated alterations in paediatric relapsed/refractory cancers.**

**A)** Columns represent 217 patients of the MAPPYACTS cohort with respective alterations in core pathway genes, growth factor pathway or *MYC/N/L* as indicated below (row names). Concomitant TP53 and RAS and *BRAF* mutation status is given at the bottom. A list for mTOR-other and RTK-other class summarizing heterogeneous genes is given in Supplementary Table 3. Tumour type is given on the top. **B)** Fraction of the MAPPYACTS cohort with contributory advanced profiling showing alterations in pathways of interest for mTOR inhibition. **C)** Distribution of genes affected by alterations in core PI3K/AKT/mTOR pathway. Abbreviations: EWS, Ewing sarcoma; OS, osteosarcoma; RMS, rhabdomyosarcoma; NRSTS, non rhabdomyosarcoma soft tissue sarcoma; WT, Wilms tumour; NBL, neuroblastoma; Other ST, other solid tumour; B-ALL, acute B lymphoblastic leukaemia; T-ALL, acute T lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; ALCL, anaplastic large cell lymphoma; other NHL, other non-Hodgkin lymphoma; HGG, high grade glioma; LGG, low grade glioma; MB, medulloblastoma; EPD, ependymoma; Other CNS, other CNS tumours.

## Discussion

Here we present a first-in-child clinical trial, treating paediatric cancer patients with the dual mTORC1/2 inhibitor vistusertib. This is, to our knowledge, the only study evaluating this drug class in children. The preliminary clinical and pharmacokinetic data indicate that vistusertib at the adult body surface area-equivalent dose is well tolerated in an intermittent schedule. The combination with chemotherapy resulted mainly in haematologic toxicity. Based on risk for DLT vistusertib at 50% of the single agent recommended dose and TOTEM at 66% of its combination dose is close to the RP2D<sup>26</sup>. The information obtained points to a toxicity profile similar to the class safety profile<sup>18</sup>.

Despite this, all arms were prematurely closed to inclusion in June 2018 following the results of two randomized studies in adults showing inferiority of vistusertib compared to everolimus<sup>19,20</sup> and the decision of AstraZeneca to stop all internal trials with vistusertib. Due to an interim-analysis of our cohort and strong signals indicating insufficient target engagement with equivalent drug dosing in adult patients<sup>20</sup>, a joint decision was taken to suspend all study arms with vistusertib. Critical in this process was to identify inefficacy based on drug intrinsic activity failure, rather than related to the indication. The following additional studies support this decision<sup>21,34</sup>. This came despite highly superior preclinical data<sup>11,12,15-17</sup> and pharmacokinetic parameters within the targeted range<sup>18</sup>, highlighting the importance of robust pharmacodynamic readouts in early phase clinical trials and the limited prediction of some preclinical findings. Until now, second or third generation mTOR inhibitors have failed to translate to the clinic due to increased side effects induced by combined mTORC inhibition with lack of superiority in clinical outcome<sup>35</sup>. The combination with TOTEM

was added due to the previous in general limited activity with single agent mTORC1 inhibitors and the potential role of PI3K/mTOR alterations in chemoresistance<sup>36-38</sup>.

In contrast to all published adult studies we had chosen to enrich both arms with patients whose tumours exhibited alterations in the target pathway. This is the core concept of the ESMART platform that investigates targeted agents in enriched cohorts based on the hypothesis that genetic alterations confer increased sensitivity. Despite molecular enrichment no indication of clinical benefit was obtained, providing additional confidence, that overall negative studies are likely related to the insufficiently active molecule. One caveat is that no patient with *MYCN*-amplified neuroblastoma was treated for which preclinical data in paediatric models were strongest<sup>13</sup>.

We want to emphasize that negative results such as these should not discourage early stage drug access for children as restriction to drugs that have shown to be active in adult cancers would not only delay access for children, but also hinder the identification of compounds preferentially active in paediatric malignancies. Inefficacy in early clinical testing is common with < 10% of molecules advancing to approval<sup>39</sup>. The ESMART platform and similar initiatives (NCI-MATCH) highlight our search for innovative trial designs to meet these needs in the area of targeted therapies. Given the small number of eligible patients in paediatric oncology, enrichment is expected to increase likelihood of positive treatment response signals allowing an efficient and safe framework to evaluate clinical and molecular hypothesis. For the presented treatment arms the mandate for a paediatric investigation plan (PIP) by the European Medicines Agency, enabled early access to vistusertib for clinical testing in children. Regulatory frameworks such as this and the close exchange with adult oncology to drive joint Phase I/II projects are in our experience crucial to foster early drug access

with its potential benefits for children. This always carries the risk of those drugs proving ineffective.

To evaluate the potential for future studies targeting this pathway, we explored our molecular profiling data from the MAPPYACTS trial in regard to alterations affecting either the PI3K/AKT/mTOR signalling effectors themselves or through activation of upstream pathways. Based on this data up to 34.9% of patients (217 of 622) in a cohort of relapsed/refractory paediatric cancers could potentially benefit from effective treatment targeting these pathways. Core pathway alterations were detected in 10.5%, with predominance of established variants such as activating mutations in *PIK3CA*, loss of tumour suppressor *PTEN* and AKT amplification. This is consistent with previous literature<sup>40</sup>. Of note, whereas a link between sensitivity to mTOR inhibitors is established for a subset of mutations<sup>41-43</sup>, stringent biomarkers for treatment response are lacking in the majority of cases and it remains to be explored whether molecular enrichment leads to an increased sensitivity to treatment.

Despite the inefficacy of vistusertib, the frequency of alterations in paediatric cancer still supports our rationale to keep on exploring how to target this key pathway in childhood malignancies. As our current knowledge points towards a role in metabolic flexibility and therapy resistance rather than being a central driver in the majority of cancers<sup>36-38</sup>, we see the future of targeting mTOR as an add-on in combination with other therapy approaches.

## **Acknowledgments**

We are most grateful to the patients and their families who contributed to this study. We thank their treating teams, the Gustave Roussy Clinical Research Direction for sponsoring the trial and AstraZeneca for providing vistusertib. We thank A Sykes for

analysis of PK data as well as Windy Rondolf and Antonin Marchais for support with R. The trial was in part developed in the Joint ECCO-AACR-EORTC-ESMO Workshop in 2014 (Francisco Bautista, BG, XP).

## **Funding**

The ESMART and MAPPYACTS trials were supported by grants from the Institut National de Cancer (INCa) through the AcSé program and the PHRC “INCa-DGOS\_8519” MERRI, the Association Imagine for Margo, and Fondation ARC, the Fédération Enfants et Santé, the Société Française de lutte contre les Cancers et les leucémies de l’Enfant et l’adolescent (SFCE), Dell and AstraZeneca. BG is supported by the ‘Parrainage médecin-chercheur’ of Gustave Roussy. RJM is supported by the Swiss National Science Foundation and the Kurt und Senta Herrmann Stiftung. LVM is supported through the Oak Foundation via the Royal Marsden Cancer Charity.

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## **Figure 1: Visualization of patient target lesion best response (top) and individual correlated genetic alterations.**

Clinically progressive disease without imaging was arbitrarily set to 100%. Rows indicate genes with respective names and columns indicate patients with Arm E / F and identifier on top. \* progressive disease due to new or increase of non-target lesions. Abbreviations: NA: not applicable; CP: clinically progressive disease; CNS: central nervous system.

## **Figure 2: Total plasma concentration and exposure of vistusertib**

A) Total plasma concentration of vistusertib monotherapy at 75 mg/m<sup>2</sup> BID in children on day 1 (N=4), given as mean ± standard deviation and B) day 9 steady state (N=2), given as mean ± range. Panel C) indicating area under the curve (AUC) exposure of vistusertib in single patients and comparison to adult AUC mean for vistusertib at 100 mg as reference. \* Adult AUC mean and range, data taken from Basu et al. 2015 <sup>18</sup>.

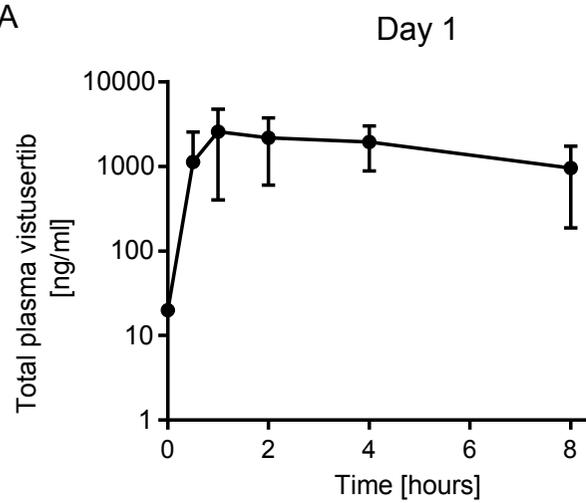
## **Figure 3: Core PI3K/AKT/mTOR-pathway and associated alterations in paediatric relapsed/refractory cancers.**

**A)** Columns represent 217 patients of the MAPPYACTS cohort with respective alterations in core pathway genes, growth factor pathway or *MYC/N/L* as indicated below (row names). Concomitant TP53 and RAS and *BRAF* mutation status is given at the bottom. A list for mTOR-other and RTK-other class summarizing heterogeneous genes is given in Supplementary Table 3. Tumour type is given on the top. **B)** Fraction of the MAPPYACTS cohort with contributory advanced profiling showing alterations in pathways of interest for mTOR inhibition. **C)** Distribution of genes affected by alterations in core PI3K/AKT/mTOR pathway. Abbreviations: EWS, Ewing sarcoma; OS, osteosarcoma; RMS, rhabdomyosarcoma; NRSTS, non rhabdomyosarcoma soft tissue sarcoma; WT, Wilms tumour; NBL, neuroblastoma; Other ST, other solid tumour; B-ALL, acute B lymphoblastic leukaemia; T-ALL, acute

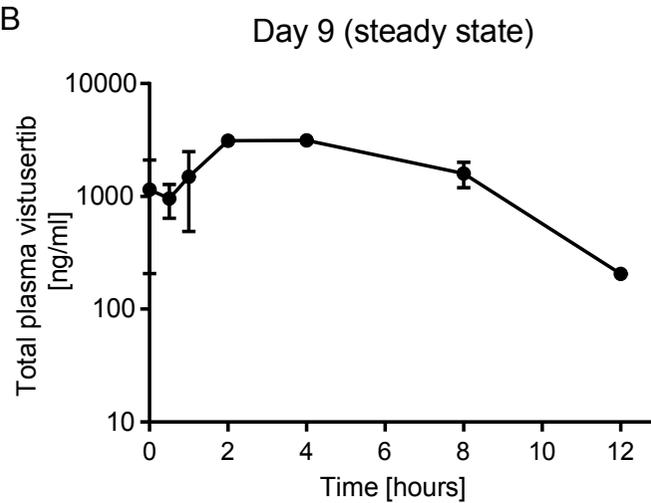
T lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; ALCL, anaplastic large cell lymphoma; other NHL, other non-Hodgkin lymphoma; HGG, high grade glioma; LGG, low grade glioma; MB, medulloblastoma; EPD, ependymoma; Other CNS, other CNS tumours.



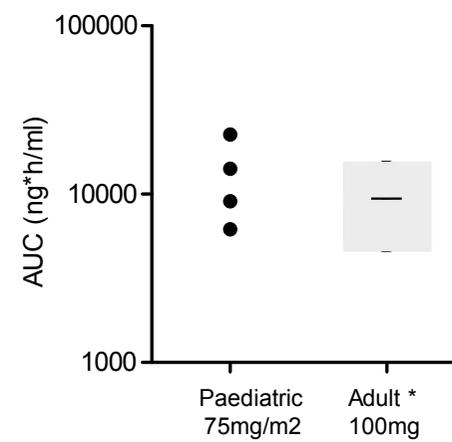
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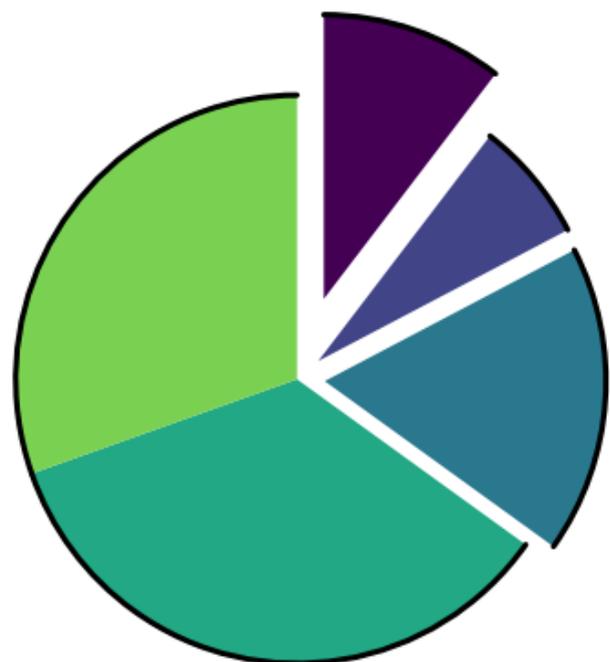
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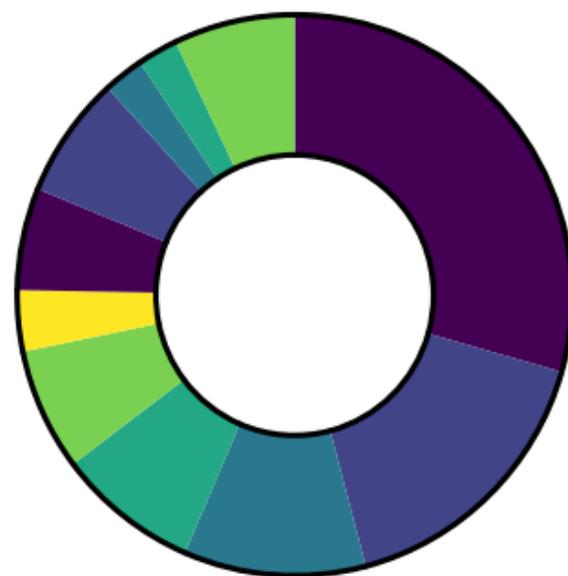
## Fraction of contributory patients



Total=622

- 65 PI3K/AKT/mTOR
- 43 MYC/N/L
- 109 Growth factor
- 216 Other alteration
- 189 No alteration

## Alterations across 65 patients



Total=85

- PTEN
- PIK3CA
- AKT1
- PIK3R1
- AKT2
- AKT3
- TSC1
- TSC2
- MTOR
- RICTOR
- others