Assessment of MGMT promoter methylation and clinical benefit from temozolomide-based therapy in Ewing sarcoma patients

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Coordinator:

Emanuela Palmerini, MD

Sezione di Chemioterapia dei Tumori dell' Apparato Locomotore Istituti

Istituto Ortopedico Rizzoli,

Bologna, ITALY

Contatti:

Emanuela Palmerini, MD

Sezione di Chemioterapia dei Tumori dell' Apparato Locomotore Istituti

Istituto Ortopedico Rizzoli,

40136 Bologna, ITALY

e-mail: emanuela.palmerini@ior.it

Tel. +39/051 6366199

FAX +39/051 6366277

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Introduction

The current treatment modalities for Ewing sarcoma (EWS), including surgery, radiotherapy and chemotherapy, significantly improved the survival of these tumors, shifting the average 5-years disease free survival rate from 10% to 65-75%. Nonetheless metastatic patients and patients with unfavorable prognostic factors (like poor pathological response, large tumor mass, multiple sites lesions) have poor survival.

Patients who are metastatic at the time of diagnosis have an expected survival < 40%, while it is <20% in case of relapse.

The standard treatment for Ewing sarcoma is based on the use of a doxorubicn, vincristin, ifosfamide, dactinomycin D, cyclofosfamide ad etoposide regimen with, in selected cases, high dose busulphan and melphalan with autologous peripheral blood stem cells rescue.

In the last years, for patients with high risk Ewing's sarcoma, the treatment with irinotecan and temozolomide has been introduced.

The activity of irinotecan (a topoisomerasis I inhibitor) and temozolomide (alkylating agent) has been study either in monotherapy and in combination, using different schedules and in combination with other drugs and target therapy.

The experience in the use of temozolomide as single agent in is limited to 1 case report: 180 or 215 mg/m2/die orally administered for 5 days in 7 pediatric patients with relapsed Ewing sarcoma. This study, with the compassionate use of temozolomide, documented a progressive disease in all the treated patients, after 1 or 2 cycle of therapy.

The actual available studies are related to patients treated with irinotecan as single agent continuously administered at low dosage, based on the pre-clinical results that demonstrated that this schedule has a minor toxicity compared to irinotecan given at high doses for a shorter time,

Memorial Sloan Kettering reported 3 Ewing's Sarcoms cases, treated with irinotecan 20 mg/m2 for 5 days, given for 2 weeks (20 mg/m2 x 5 x 2) every 3 weeks. All the patient had progressive disease (the time for the response evaluation is not reported).

St Jude's Hospital presented a Phase I dose finding study which included 2 patients with Ewing's Sarcoma that were treated with irinotecan up to 45 mg/m2 for 5 days given for 2 weeks. One of the cases, reported a Partial Response (PR) that lasts for 6 cycles. Another St Jude's study of 3 Ewing's Patients treated with of irinotecan 5-20 mg/m2 for 5 days for 2 weeks, and Gefitinib orally administered, documented 1 PR lasted for 4 cycles.

The most interesting study on the use of irinitocan in metastatic Ewing's Sarcomas as single agent, is the phase II window study, in patients enrolled in EuroEWING99 protocol, and receiving ifosfamide 600/m2, single infusion, every 3 weeks. Five out of 22 patients included had a PR, 1 patient had minimal response and 9 had SD.

Combination studies were based on sinergic activity of irinotecan, when combined with temozolomide; this effect is dose dependent.

Four irinotecan plus temozolomide in relapsed Ewing sarcoma studies were published, as reported in Table 1. In all studies irinotecan was given in prolonged infusion, at a low dosage, with a 4-fold irinotecan dose difference, in a total number of 65 patients.

In the first phase I study 14 patients were evaluable, reporting 1 CR, 3 PRs, and 4 SDs.

In a retrospective series from MDACC in 25 patients, 7 CRs, 9 PRs, and 6 SDs were documented. The MSKCC reported 5 CRs, and 7 PRs, as "best responses" out of 19 patients. The Nino Jesús Pediatric Hospital, in Madrid, documented 3 PRs and 2 SDs out of 7 patients.

Combining these 4 series, the 65 included patients ha a response rate (RR) of 54% and a median time to progression (mTTP) ranging from 4.6 to 8.3 months. The progression free

survival was not reported. Three groups reported a total of 31 patients treated with vincristine in combination with temozolomide and irinotecan, with 4 differrent schedules, inlcluding irinotecan per os (see Table 1).

The larger study is a polish retrospective series in 22 cases, reporting 5 CRs, 7 PRs and 3 SDs, for a RR of 55%.

Oral irinotecan was employed at Cincinnati Children's Hospital, in different trials. In the 1st study 1 unconfirmed CR, 1 PR and 1 SD were documented in 5 patients. In the second, with the addition of bevacizumab 15 mg/kg on day 1, 2 Ewing cases were included, with 1 CR after 6 cycles and 1 PR after 6 cycles.

Table 1

Authors	TMZ (mg/m2)	Irinotecan (mg/m2)	Other agents	Responses
Wagner	100 x 5	IV 10-20 x 5 x 2	None	4
Anderson	100 x 5	IV 10 x 5 IV 10 x 5 x 2	None	16
Casey	100 x 5	IV 20 x 5 x 2	None	12
Hernandez-Marques	80-100 x 5	IV 10-20 x 5 x 2	None	3
Raciborska	125 x 5	IV 50 x 5	Vincristine x 1	12
McKnall-Knapp	100 x 5	IV 20 x 5 x 2	Vincristine x 1	1
Wagner	100-150 x 5	Orale 35-90 x 5 Orale 35-90 x 5 x 2	Vincristine x 1 Vincristine x 2	2
Wagner	150 x 5	Orale 90 x 5	Vincristine x 1 Bevacizumab	2

Toxicity of irinotecan temozolomide combination is mainly hematological and gastrointestinal. Even in heavily pretreated patients, infrequent were G 3-4 thrombocytopenia and neutropenia. Grade 1-2 diarrhea was reported after about 50% of cycles, nonetheless in only 10% of patients reached grade 3-4, rarely requiring treatment discontinuation.

The Cincinnati study suggests that grade ≥3 diarrhea is more common when irinotecan is given at a dose > 10 mg/m2/die.

Other side effects included nausea and vomiting, up to 15% of the patients, and 2 cases of grade 3-4 of hepatic toxicity.

Temozolomide is an alkylating agent and recent studies demonstrated a key role of O6-methylguanine-DNA methyltransferase (MGMT) enzyme in mechanisms of resistance to this class of drugs.

.Epigenetic silencing of the MGMT (O6-methylguanine-DNA methyltransferase) DNA repair gene by promoter methylation has been associated with longer survival in TMZ treated glioblastoma patients, and we believe that methylation studies could identify the most appropriate population to treat.

No studies on MGMT methylation status have been reported in patients with Ewing sarcoma.

Given these considerations and due to the demonstrated efficacy of irinotecan / temozolomide combination in Ewing, we propose to assess MGMT methylation status in metastastic Ewing patients treated with temozoloide and irinotecan, in order to identify the most responsive patients.

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Study Objectives

- Primary Endpoint :

Assessment of MGMT promoter methylation and clinical benefit from temozolomide-based therapy in Ewing sarcoma patients

- Secondary Endpoint:

Correlation of the MGMT promoter methylation to the activity of the temozolomide + irinotecan combination

Trial Design

This is an observational retrospective and prospective study on the use of temozolomide and irinotecan in patients treated at SSD Chemioterapia of Istituto Ortopedico Rizzoli (Bologna Italy) and in other Italian and European Hospitals

The MGMT methylation status will be correlated with the disease clinical data and with the disease response in term of RECIST 1.1 response (Table 2). If available the metabolic response will be evaluated with PET TB (Table 3)

The MGMG methylation analysis will be done in collaboration with the following laboratories

- Research Unit "Molecular Characterization of Sarcoma",
- CRS Development of Biomolecular Therapies Experimental Oncology Lab,

The test will be performed extracting from fresh tumor sample or from FFPE (based on the availability) , the DNA on the basis of the standard protocols. The MGMT methylation will be evaluated by the EpiTect Bisulfite analysis according the following steps:

- Target DNA will be incubated at high temperature and low pH with sodium bisulfite. Bisulfite conversion, transforms cytosine residues into uracil residues but leaves 5-methylcytosine residues unchanged
- The bisulfite converted DNA, will be then transferred into EpiTect spin column, when it will be eluted and purified

- The purified converted DNA, will be amplified by a DNA methylation-specific polymerase chain reaction (MSP) with primers specific for MGMT.

- After amplification the MSP-PCR products will be electrophoresed on a 2% agarose gel and will visualized with *UV* light .

All the patient's data will collected in anonymized way and will be managed and retained by the SSD Chemioterapy ward of Istituto Ortopedico Rizzoli Clinical Trial Office.

The complete list of the collected data is reported into the Case Report Form (pg 12)

Subject Selection

Inclusion Criteria

- Histological confirmed diagnosis of Ewing's Sarcoma
- Chemotherapic treatment with temozolomide and irinotecan
- Written informed consent prior to any study related activities

Exclusion Criteria

-Lack of written informed consent for the study
-Any situation that could interfere with the complete data collection of the clinical data related to the temozolomide and irinotecan treatment

Enrollment

The expected duration of this study is 36 months. An extension of this period could be evaluated if a correlation between the MGMT methylation status and temozolomide/irinotecan activity, will be observed and thus a sample size increase will be required.
The study will be terminated if the first consecutives 15 patients will not present any alteration of the MGMT methylation status.

Sample Size and Statistic

On the basis of the incidence of the disease, the expected number of analysis will be 15/yr for the retrospective and 9/yr for the retrospective part of the study.
The MGMT methylation status will be evaluated on 42 samples.
Due to the lack of literature data it is not possible to predict the incidence of the expected MGMT methilation status in the study sample.

If the first consecutives 15 patients will not present any alteration of the MGMT methylation status, the study will be early terminated.

If alteration of the MGMT methylation will be observed the data will be analyzed (χ-square test) so to correlate the presence and the absence of methylation with the radiological response variables based on RECIST 1.1 criteria (Complete Response –CR-, Partial Response – PR- and Stable Disease – SD-).

If this analysis will result in a possible correlation between the MGMT methylation status and the radiological response, a new prospective study will be developed on the basis of the results of this research.

Due to the observational design of the study the sample size is not pre-determined and only the duration of the study can be estimated.

Ethic and logistical aspects

This project has been awarded and received the financial support from *"Bando per la ricerca Regione-Università, Alessandro Liberati*".

All the financial aspect of this research will be covered by *"Progetto di ricerca Regione-Università, Alessandro Liberati (Progetto PRUA1GR-2013-00000114*)", Principal Investigator: Dr. Emanuela Palmerini.

Tabella 1: RECIST 1.1

Target	Non-target	New	Overall Response
CR	CR	No	CR
CR	Non-CR / non-PD	No	PR
CR	NE	No	PR
PR	Non-PD or NE	No	PR
SD	Non-PD or NE	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Response criteria

Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions.

Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sumdiameterswhile on study.

Evaluation of non-target lesions

While some non-target lesions may actually be measurable, they need not bemeasured and instead should be assessed only qualitatively at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalisation of tumour marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression of existing non-target lesions.

Reference:
E.A. Eisenhauera,, P. Therasseb, J. Bogaertsc, L.H. et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1) EUROPEAN JOURNAL OF CANCER 45 (2 0 0 9) 2 2 8 –2 4 7

Table 2: PERCIST: Positron Emission Tomography Response Criteria in Solid Tumors

Response category	Criteria
Complete metabolic response	Normalization of all lesions (target and nontarget) to SUL less than mean liver SUL and equal to normal surrounding tissue SUL
	Verification with follow-up study in 1 month if anatomic criteria indicate disease progression
Partial metabolic response	> 30% decrease in SUL peak; minimum 0.8 unit decrease*
	Verification with follow-up study if anatomic criteria indicate disease progression
Progressive metabolic disease	> 30% increase in SUL peak; minimum 0.8 unit increase in SUL peak*
	> 75% increase in TLG of the 5 most active lesions
	Visible increase in extent of FDG uptake
	New lesions
	Verification with follow-up study if anatomic criteria indicate complete or partial response
Stable metabolic disease	Does not meet other criteria

Primary outcome determination is measured on the single most active lesion on each scan (not necessarily the same lesion).
Secondary outcome determination is the summed activity of up to 5 most intense lesions (no more than 2 lesions per organ).

Abbreviazioni: SUL, standardized uptake value using lean body mass; TLG, total lesion glycolysis.

Reference:
Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med. 2009;50(Suppl 1):122S-150S