O6-methylguanine-DNA methyltransferase (MGMT) status in neuroendocrine tumors: a randomized phase II study (MGMT-NET)

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TITLE PAGE

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Abstract

Introduction: Neuroendocrine tumors (NETs) are rare, but their incidence is rising. Alkylating agents (ALKY), temozolomide and streptozotocin, are the main chemotherapies used for advanced pancreatic NETs. According to retrospective data, O6-methylguanine-DNA methyltransferase (MGMT) status appears to be a predictive factor of the response to ALKY.

Aims: The main objective is to evaluate the value of tumor *MGMT* promoter (*pMGMT*) methylation in the prediction of the objective response (OR) at 3 months in patients treated with ALKY. Secondly, we will evaluate the value of MGMT immunohistochemistry and the efficacy of treatment with ALKY *vs*. oxaliplatin-based chemotherapy (Ox).

Materials and methods: A national, prospective, open-label, randomized, controlled and multicenter trial was designed. Main inclusion criteria are: adult patients with well-differentiated advanced duodeno-pancreatic, lung, or unknown primitive NETs with a validated indication for chemotherapy. *pMGMT* methylation will be assessed by pyrosequencing, but an ancillary study will compare this technique with others ones including MGMT immunohistochemistry.

Results: A total of 104 patients will be randomly assigned (1:1 for unmethylated or 2:1 for methylated *pMGMT* NETs) to either the ALKY arm or to the Ox arm.

Conclusion: Recruitment started on October 16, 2018 (NCT03217097) and will be open in 21 centers in France.

Keywords: Neuroendocrine; Clinical trial; O6-Methylguanine-DNA methyltransferase; Alkylating agents

1. Rational and aims

Neuroendocrine tumors (NET) are rare, but their incidence is rising and their prevalence is high (1). Most occur in the digestive system (68%) and the bronchopulmonary system (25%), and more than 60% are diagnosed at advanced, unresectable stages (1). Chemotherapy is one of the few therapeutic weapons, along with targeted therapies, somatostatin analogs, and metabolic radiotherapy. Alkylating agents (ALKY), temozolomide and streptozotocin, are one of the main systemic treatments used (2-4), at least for advanced duodeno-pancreatic NETs; the response rate is 30% to 40% and the median progression-free survival is 4 to 18 months (2, 5-9). However, it should be noted that for pulmonary NETs, called typical and atypical carcinoids, the level of proof of efficacy of these treatments is lower than for duodeno-pancreatic NETs.

One of the mechanisms of ALKY cytotoxicity is the induction of DNA alkylation/methylation at O6-guanine sites, resulting in DNA mismatch and cell death in tumor tissue (10). However, ALKY-induced DNA damage can be repaired by O6-Methylguanine-DNA methyltransferase (MGMT). Any reduction in MGMT activity may therefore potentiate the effect of ALKY. The expression of MGMT has been shown to be decreased in some tumor cells, mainly as a result of gene promoter hypermethylation (11). Thus, MGMT status has been proposed as a predictive factor for the response to ALKY; it can be assessed at the protein level (by immunohistochemistry, IHC) and at the gene level (through methylation analysis). The current literature is conflicted as to the value of MGMT to predict response to ALKY, in part because MGMT status is assessed by multiple techniques with various accuracy, but also because of the retrospective nature of the studies reported and the low number of patients included (12-21) (Table 1).

In glioblastoma, methylation of *pMGMT* is predictive of the efficacy of temozolomide with an increased survival (22-25). As in glioblastoma (24, 26-28), *pMGMT* methylation assessed by

pyrosequencing (with immunochemistry, IHC) is the most effective and reproducible technique to predict the response to ALKY in NETs (13). *pMGMT* is methylated in 25%-50% of NETs in general, but it should be noted that a variation exists depending on the site of primitive cancer. Current data estimate a methylation of *pMGMT* in about 50% of pancreatic NETs and 0%-15% of lung and gastro-intestinal NETs (12, 13, 17). This variation could also contribute to the different chemosensitivities of these tumors. To the best of our knowledge no prospective study investigating MGMT status in NET has been published; currently, two studies are registered in ClinicalTrials.gov that evaluates MGMT as a secondary endpoint (NCT02698410 and NCT01824875). The latter was recently presented by Kunz *et al*. at the ASCO 2018 annual meeting, but results regarding MGMT are currently pending (9).

Furthermore, although ALKY are commonly recommended upfront in NET (2-4), oxaliplatin (Ox), either with 5-fluorouracile (29-35) or with gemcitabine (33, 36), has shown interesting activity with response rates ranging from 17% to 30%. In a retrospective study, we demonstrated that GEMOX is effective in NET and that its activity is similar to ALKY, but irrespective of the MGMT status (16). Prospective studies are needed, but the data suggests that ALKY should be offered first to patients with *pMGMT* methylation while Ox-based chemotherapy should be offered first to patients with unmethylated *pMGMT* tumors.

In this context, the purpose of this prospective study is to evaluate the contribution of the *pMGMT* methylation, in predicting the objective response (OR) in patients treated with ALKY and to evaluate a treatment with ALKY versus Ox in patients with a NET.

2. Study design

The MGMT-NET study is a national, prospective, open-label, randomized, controlled and multicenter trial assessing the value of using the MGMT status in clinical practice to predict OR of patients suffering from NET. Patients included will first undergo analysis to determine

the MGMT status before randomization. Patients with unmethylated *pMGMT* NETs will be randomly assigned (1:1) to either the ALKY-based chemotherapy arm or to the Ox-based chemotherapy arm. Patients with methylated *pMGMT* NETs will be randomly assigned (2:1) to either the ALKY-based chemotherapy arm or to the Ox-based chemotherapy arm (Figure 1). As current data suggests that patients with methylated *pMGMT* respond better to ALKY (12, 13) we considered that a 1:1 randomization would not have been ethical for such patients. In addition, an Ox arm was included to facilitate recruitment by offering another option of treatment to patients. However, we considered that a phase III study designed to evaluate the best chemotherapy (Ox vs. ALKY) according to MGMT status in each arm would have been impossible to complete given the high number of patients needed and the rarity of the disease.

This study includes patients aged 18 years or above, with a well-differentiated NET (duodeno-pancreatic, lung, or unknown primary site), grades 1 to 3, metastatic or locally advanced, and not curatively operable. Systemic chemotherapy must be indicated and validated by a multidisciplinary tumor board. Previous local or systemic treatments other than ALKY or Ox -based chemotherapy are allowed. Tumor tissue should be available for the patient to be included in order to investigate MGMT status. The main exclusion criteria are: previous chemotherapy using Ox or ALKY, and contraindication to any ALKY drugs or oxaliplatin. All patients must have given their written consent for participation in this trial.

Methylation tests and IHC will be centralized and conducted on the most recent available histological material. *pMGMT*_methylation status will be evaluated using two techniques: methylation-specific polymerase chain reaction (MS-PCR) and pyrosequencing, which will provide, respectively, qualitative and quantitative information. The techniques used are derived from the procedures employed in our institution for gliomas. All examined samples must contain more than 80% tumor cells. DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue is performed after deparaffinization using a purification kit

(Promega, Madison, WI, USA, ref AS1450). Genomic DNA is modified by bisulfite conversion (EZ DNA Methylation Gold Kit, Zymo, Irvine, CA, US). For MS-PCR, we use the primers described by Dong *et al*. (37). For pyrosequencing analysis, an 8% cutoff is used (26). *pMGMT* promoter is therefore scored "methylated" if more than 8% methylated alleles are detected compared to unmethylated alleles. The results are presented as methylated, not methylated, or not interpretable. The MGMT protein expression will be evaluated by an immunohistochemical technique for which an automated immunostaining system will be used (Ventana Benchmark, Tucson, AZ, US). MGMT expression will be assessed on a whole slide using a score based on nuclear staining intensity (0-3) multiplied by the proportion of stained cells (0-100%). The score ranges from 0 to 300 and tumors are considered MGMT negative (loss of expression) if the score is ≤50 (17). *pMGMT* methylation test and IHC will be carried out in parallel, but only the result of the methylation test will be considered for randomization unless the result is not interpretable; in which case the IHC result will be used, considering that a loss of MGMT expression in IHC corresponds to a methylated *pMGMT*. If the *pMGMT* methylation test and the IHC result are both not interpretable, the patients will be randomized (ratio 1:1 between the two arms), but these patients will not be included in the analysis of the main outcome. Furthermore, a blood sample will be collected specifically for circulating free DNA (cfDNA) analyses.

Taking a pragmatic approach with a view to complete the study and because chemotherapy regimens in NETs are not standardized, we do not impose a specific regimen in both arms. However, one regimen in each arm is recommended in order to reduce heterogeneity: the ALKY-based chemotherapy arm will receive capecitabine (750 mg/m^2 twice daily for 14 days, days 1-14) and temozolomide (temozolomide, 200 mg/m^2 once daily for 5 days, days 10-14), every 28 days) (3, 9), alternatively LV5FU2-dacarbazine (for patients with expected difficulties with adherence to oral chemotherapy) or 5FU-streptozotocine (only approved in

France for pancreatic NET, therefore only used in this study in this situation). The recommended regimen in Ox-based chemotherapy arm is GEMOX (gemcitabine 1000 mg/m^2 followed by oxalipatlin 100 mg/m^2 every 2 weeks) (16); alternatively FOLFOX or CAPOX. Doses and monitoring of chemotherapies will be administered in accordance to French recommendations (38). The duration of chemotherapy recommended is at least 3 months (assessment of the primary endpoint), but physicians are allowed to continue chemotherapy, and do so usually for 4-12 months (2, 5-9).

The radiological assessment will be performed at baseline (within a maximum of 4 weeks before inclusion) using a computed tomography (CT) scan and/or magnetic resonance imaging (MRI), and the same procedure (CT or MRI) will be repeated at 3 months after the beginning of treatment to enable radiological tumor assessment according to the RECIST v1.1 criteria.

2.1 Study endpoints

The primary endpoint is the 3-month OR rate (complete response – CR, or partial response – PR) assessed according to the RECIST v1.1 criteria and based on a centralized reviewing performed by an expert radiologist blinded to the result of *pMGMT* methylation and treatment assignment.

The secondary endpoints are to evaluate and compare the progression-free survival (PFS) assessed by RECIST v1.1 criteria and overall survival (OS) according to *pMGMT* methylation in patients treated with ALKY; the OR at 3 months, PFS, and OS according to *pMGMT* methylation in patients treated with Ox-based chemotherapy; and to evaluate the value of the MGMT status for predicting OR at 3 months as assessed by RECIST v1.1 criteria based on MGMT status evaluated using immunochemistry. In addition, we will evaluate in tumor tissue and in cfDNA if possible, certain alterations previously described in pancreatic or lung NET,

such as DAXX/ATRX, mTOR pathway (PTEN, TSC2), MEN1, ARID, SMAD, VHL (39); their prognostic value in NET as assessed by RECIST v1.1 criteria will be explored.

2.2 Ethical considerations

This study is sponsored by the Hospices Civils de Lyon and was authorized by the French medicines agency (*Agence Nationale de Sécurité du Médicament et des produits de santé*, ANSM) on June 27, 2018. It was submitted and approved (August 1, 2018) by the institutional review board (*Comité de protection des personnes*).

This trial is registered on the clinicaltrials.gov website (NCT03217097). The study complies with the Declaration of Helsinki and the principles of Good Clinical Practice guidelines.

2.3 Statistical methods

The primary endpoint is the OR rate at 3 months among patients treated with ALKY according to *pMGMT* methylation status. OR will be compared between the two groups using a Chi-square test. The response rate and the associated 95% confidence interval (CI) will be provided for each group. The sample size calculation was calibrated to detect a 35% absolute difference in the OR at 3 months according to *pMGMT* methylation among patients treated with ALKY. This translates into an improvement in OR that ranges from 15% in patients with unmethylated *pMGMT* NETs to 50% in patients with methylated *pMGMT* NETs. This calculation is based on an expected number of 55 patients treated with ALKY (i.e. 22 patients with a methylated *pMGMT* NETs and 33 patients with an unmethylated *pMGMT* NETs) to obtain a 75% power to show a statistically significant ORR difference with a one-sided α risk of 5%. Considering the hypothesis that a third of the patients will have a methylated *pMGMT* tumor, 99 patients need to be randomized in the study. However, assuming that 5% of the patients will not be assessable for *pMGMT*, we plan to include 104 patients for *pMGMT*

methylation, and 99 patients will be randomized. The randomization will be conducted according to *pMGMT* methylation and stratified by the location of NET. PFS and OS will be estimated using the Kaplan-Meir method according to MGMT status and NET location. The first patient was included on October 16, 2018.

Conflicts of Interests

None declared

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Legend of Figure

Legend of figure 1: Study design. The methylation test and the IHC are carried out in parallel, but only the result of the methylation test is considered unless the result is not interpretable, in which case the IHC result will be used.

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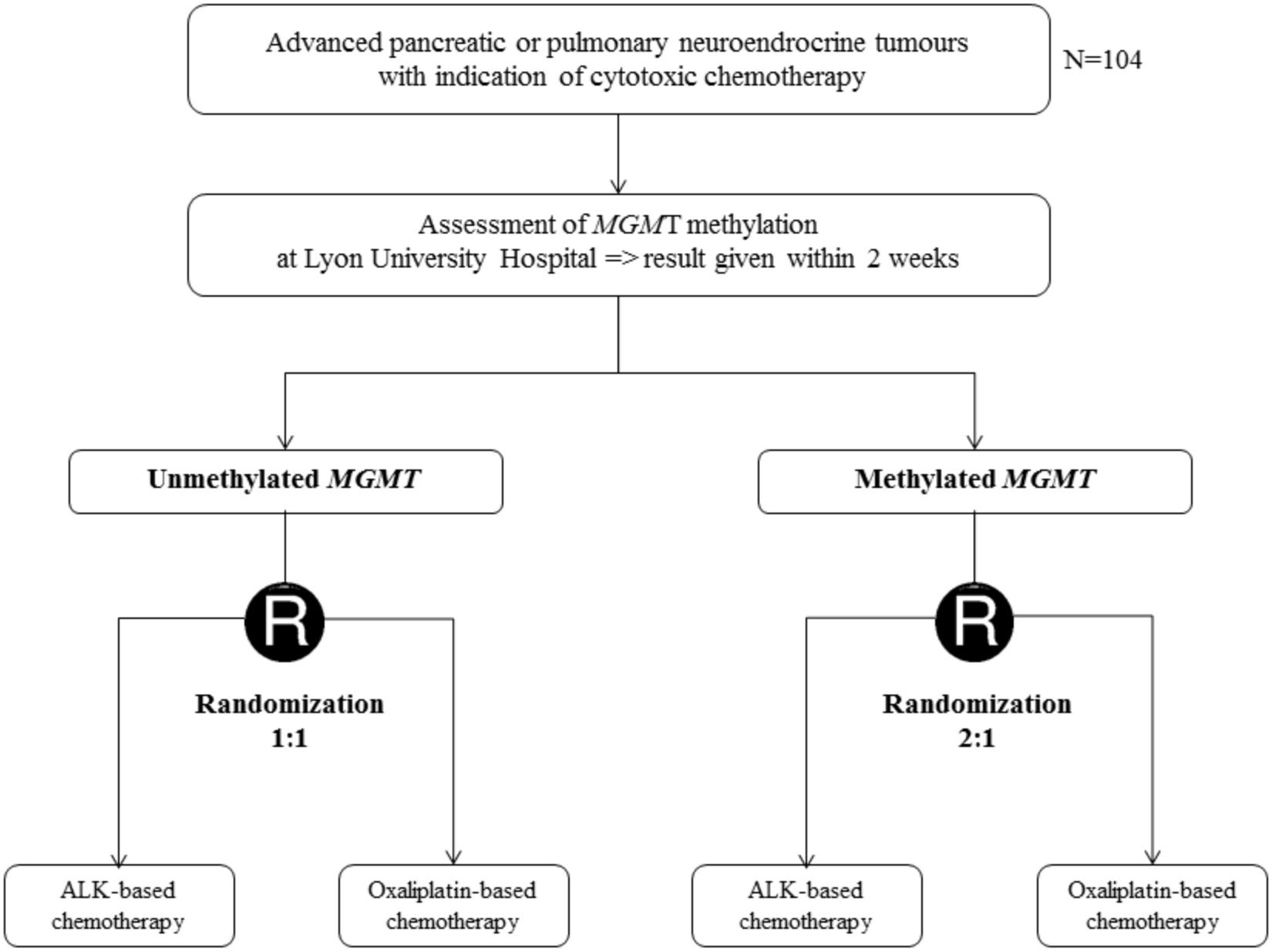
Advanced pancreatic or pulmonary neuroendrocrine tumours with indication of cytotoxic chemotherapy
N=104
Assessment of *MGMT* methylation at Lyon University Hospital => result given within 2 weeks
Unmethylated *MGMT*
Methylated *MGMT*
R
R
Randomization 1:1
Randomization 2:1
ALK-based chemotherapy
Oxaliplatin-based chemotherapy
ALK-based chemotherapy
Oxaliplatin-based chemotherapy

Table 1. Main retrospective studies (n> 20 patients) in NETs evaluating response to alkylating agent-based chemotherapies according to the MGMT status.

	Number of treated patients	Best OR: MGMT deficient vs. not		PFS in months: MGMT deficient vs. not	
		MGMT status assessed by IHC	*pMGMT* methylation	MGMT status assessed by IHC	*pMGMT* methylation
Ekeblad 2007 (15)	23	40% vs. 8%	NA	NA	NA
Kulke 2009 (12)	21	80% vs. 0%*	NA	19 vs. 9*	NA
Walter 2015 (13)	69	62% vs. 7%*	50% vs. 11%*	20 vs. 8*	26 vs. 11*
Dussol 2015 (16)	26	38% vs. 9%	40% vs. 6%*	16 vs. 7	24 vs. 7*
Cros 2016 (17)	43	50% vs. 15%*	NA	23 vs. 11*	NR vs. 20*
Cives 2016 (18)	52	40% vs. 65%	NA	15 vs. 17	NA
Raj 2017 (20)	36	50% vs. 31%	25% vs. 38%	NA	NA
Girot 2017 (21)	22	15% vs. 11%	0% vs. 17%	18 vs. 9	18 vs. 15
Campana 2018 (19)	95	NA	52% vs. 18%*	NA	21 vs. 8*

IHC, immunochemistry; NA, not available; NR, not reached; OR, objective response; PFS, progression-free survival

* $p < 0.05$