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MGMT methylation assessed by methyl-BEAMing technique is a prognostic and predictive biomarker in glioblastoma and metastatic colorectal cancer patients

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Background: O6-methylguanine DNA methyltransferase (*MGMT*) silencing by promoter methylation is a common alteration found in different cancer types. This has been shown to be both a prognostic and a predictive marker of sensitivity to alkylating agent-based therapy like dacarbazine and temozolomide in glioblastoma. However in other malignancies its value remains controversial. This might be due to sampling issues, tumor heterogeneity or to the use of inadequate detection methods. In this study, we present a new assay to reliably measure *MGMT* methylation both in tumor and plasma samples.
Material and Methods: Methylation of *MGMT* has been assessed by an ultra-sensitive digital PCR technique, in which a two-step PCR is followed by detection via fluorocytometer (Methyl-BEAMing). Results were compared to two other commonly used techniques (Methylation Specific PCR, MSP and pyrosequencing). Two samples datasets have been evaluated: tumors from a cohort of 98 newly diagnosed glioblastoma patients from the pre-temozolomide era, and specimens from a cohort of 68 metastatic colorectal cancer patients treated with dacarbazine in a phase II clinical trial (DETECT-01 trial, EUDRACT number 2011–002080−21). The prognostic and/or predictive value of *MGMT* methylation has also been evaluated. As a proof of concept, the three methods were assessed in a subset of colorectal cancer patients' plasma derived DNA to evaluate their performance as a liquid biopsy test.
Results: Methyl-BEAMing showed high reproducibility across independent experiments, as well as high sensitivity (up to 0.09% methylation detected) and specificity. In the glioblastoma cohort, Methyl-BEAMing methylated status (>50%) was associated with a decreased hazard ratio for death (HR = 0.35; $p < 0.0001$) compared to MSP (HR = 0.54; $p = 0.006$) or pyrosequencing (HR = 0.61; $p = 0.059$). In mCRC, tissue where tumor heterogeneity is possibly higher, both Methyl-Beaming and pyrosequecing assays provided better prediction of objective response to dacarbazine than MSP. Progression free survival was also improved in metastatic colorectal cancer with methylated status when samples were assessed with Methyl-BEAMing ($p = 0.0012$) or pyrosequencing ($p = 0.0005$). Quantitative evaluation of *MGMT* methylation in circulating tumor DNA was effective with Methyl-BEAMing.
Conclusions: *MGMT* methylation testing based on BEAMing technology outperforms commonly used methods and might allow the non-invasive follow-up of patients, upon alkylating agent treatments using blood circulating DNA.

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Low, frequent doses of PM060184 induce remarkable in vivo antitumor activity

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Background: PM060184 is a synthetic marine-derived compound originally isolated from the marine sponge *Lithoplocamia lithistoides*. PM060184 induces disorganization and disruption of the microtubule network as well as aberrant mitotic spindle multipolarization and chromosome missegregation. These effects give rise to prometaphase arrest and formation of multinucleated cells. Then, cells enter to caspase-driven apoptosis or are arrested in a pseudo-senescent state. PM060184 is currently under evaluation in Phase I clinical studies in patients with advanced cancer diseases. The objective of the present work was to explore the in vivo anticancer efficacy of PM060184 administered at low daily doses (0.5 to 2 mg/kg).
Material and Methods: Athymic female *nu/nu* mice were subcutaneously implanted with different tumors: MDA-MB-231 (breast), H460 (NSCLC) and several pancreas patient-derived (AVATAR) tumors, namely JH-010, JH-015, Panc-291, and Panc-039. Tumor (*ca.* 300 mm^3) bearing animals ($N = 6-10$/group) were randomly allocated to receive PM060184 or placebo. Treatments (0.5 to 2 mg/kg, iv) were administered daily for 20 consecutive days. Antitumor effect was calculated using DT/DC (%), defined as a percentage of the change in tumor size for treated (T) and placebo (C) groups during the placebo-treated survival time (D). Complete tumor regression (CR) was defined when tumor volume <63 mm^3 for 2 or more consecutive measurements.
Results: The treatment with PM060184 produced lowest DT/DC values as summarized in the table.

Tumor		Daily dose (mg/kg)	Minimal DT/DC (%)	On Day
Breast	MDA-MB-231	2.0	7.3	21
		1.0	14.1	21
		0.5	24.0	21
NSCLC	H460	2.0	5.5	11
		1.0	7.8	11
		0.5	17.7	11
Pancreas	JH-024	2.0	19.7	21
	JH-010	2.0	14.8	21
	JH-015	2.0	38.9	28
	Panc-291	2.0	12.0	24
	Panc-039	2.0	6.1	28

Conclusion: The treatment with PM060184 at low, frequently given doses demonstrated significant in vivo antitumor activity in breast, NSCLC and pancreas xenografted tumors.

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Radiosensitizing effect of sodium metaarsenite in a metastatic brain tumor model

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Background: Brain metastases are found in about 10% of lung cancer patients at the time of diagnosis, and about 40% of all lung cancer patients develop brain metastases during their disease progression. The chemotherapy is limited because of little or no effectiveness due to the blood–brain barrier. The radiation therapy is the most frequently used, and sensitizing agents, which synergize with radiation, can improve the efficacy of the therapy.
Material and Methods: Sodium metaarsenite (KML001®) is an orally bioavailable arsenic compound that has entered phase I/II clinical trials in solid tumors and hematopoietic malignancies. In this study, we elucidated the radio-sensitizing effect of sodium metaarsenite (KML001®) in an animal model of metastasis of lung cancer to the brain.
Results: The clonogenic assay showed that treatment with sodium metaarsenite (KML001®) inhibited clone formation in radio-sensitive (H23) and radio-resistant lung cancer cells (A549 and PC14PE6) in a concentration-dependent manner. The combined irradiation and sodium metaarsenite (KML001®) treatment significantly reduced colony formation in H23 ($p < 0.01$), A549 ($p < 0.05$) and PC14PE6 lung cancer cells ($p < 0.05$), compared with the radiation alone group.
In the metastatic brain cancer model with H23 cells, sodium metaarsenite (KML001®) treatment (5 mg/kg/day) and radiation therapy (5 Gy) showed 54.5% and 67.6% reduction in tumor volume, respectively, compared with control group ($p < 0.001$ vs. control). The combined irradiation and sodium metaarsenite (KML001®) treatment induced 88.1% decrease in tumor volume ($p < 0.001$ vs. control).
In the metastatic brain cancer model with PC14PE6 cells, the single irradiation (15 Gy) and the combined irradiation (15 Gy) and sodium metaarsenite (KML001®) treatment (7 mg/kg/day) significantly increased median survival day of the mice to 22 and 26 days, respectively, compared to control group (median survival day = 19) ($p < 0.001$). The combination improved survival significantly with regard to the radiation only group ($p < 0.001$).
Conclusions: This study demonstrated that sodium metaarsenite (KML001®) may have potential as an alternative therapeutic agent,