Results: After dichotomization, preoperative blood was EpCAM-positivity (+) in 10/40 (25%) patients versus 27 (65%) patients in postoperative blood ($p < 0.0001$). EpCAM(+) in blood was seen at POD1, POD7 and after 6 weeks in 10 (28.6%), 9 (23.1%) and 8 (23.5%) patients respectively. Preoperative peritoneal lavage fluid was EpCAM(+) in 4 (10.3%) versus 21 (53.8%) patients postoperatively ($p < 0.0001$).
At none of the time-points, an association was found between EpCAM positivity in blood and/or peritoneal cavity and cancer-specific and disease-free survival. Also, no significant associations were found between clinicopathological variables and perioperative EpCAM positivity.
Conclusion: Despite a significant increase in tumor cell counts quantified with real-time RT-PCR for EpCAM, the detection of perioperative tumor cell dissemination does not seem to be associated with worse prognosis in PDAC.
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Bcl-2 family protein expression in navitoclax-treated patients (pts) with lymphoid malignancies

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Background: Navitoclax is a novel BH3 mimetic that binds with high affinity and inhibits antiapoptotic Bcl-2 family proteins. Navitoclax displays potent preclinical mechanism-based toxicity against CLL & lymphoma cell lines. These cell lines were used to generate a sensitivity and resistance profile based on Bcl-2 family protein expression. The aim of this study was to determine if this profile could act as a predictor of pt response to navitoclax.
Methods: Navitoclax activity was assessed in an ongoing phase 1 study in pts with lymphoid malignancies. Leukemia and lymphoma cell lines were used to develop and validate the expression of Bcl-2, Bcl-$_{XL}$, BIM & Mcl-1 proteins in cellular sub-populations by flow cytometry. Intensities of directly-labeled antibodies were quantified for absolute values by molecules of equivalent soluble fluorophore beads or an equivalent. The Bcl-2 family member expression was assessed in bone marrow derived lymphoma cells identified by cell surface markers using flow cytometry and in archived lymph node biopsies by immunohistochemistry.
Results: Bcl-2 & Mcl-1 protein expressions were compared in 16 pts who had both lymph node & bone marrow samples. The levels of Bcl-2 detected by either technique were in concordance, or, were similar (i.e., moderate rather than strong), in 93% of the cases. Mcl-1 levels were lower in the bone marrow, demonstrating greater variability between the measurements with concordance and similarity in only 62% of pts. The flow cytometry assay allowed comparison of Mcl-1 protein levels obtained from pt bone marrow samples and leukemic cell lines; no pts had Mcl-1 levels equivalent to the Mcl-1 levels observed in the resistant cell lines. In contrast, there were correlations between the Mcl-1 levels in the lymph node biopsies and median progression-free-survival (moderate [n = 10] 46 d, weak [n = 5] 424 d, negative [n = 2], not reached; [$p \leqslant 0.008$]).
Conclusions: A flow cytometry assay was developed to analyze the Bcl-2 family in bone marrow samples and to compare the results obtained to immunohistochemistry analysis of lymph node biopsies. Mcl-1 levels in the lymph nodes were predictive of progression-free-survival with higher Mcl-1 associated with shorter progression-free-survival in this limited data set. In contrast, the low levels of Mcl-1 observed in the pt bone marrow samples did not demonstrate a similar resistance profile, perhaps indicating differences in the tumor cells within the tissue compartments and/or the sensitivities of the methods.

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A single FISH procedure for the detection of tumor cells: Bladder cancer as model

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Background: In the present research we developed a suspension FISH (FISH-S) protocol for the identification of tumor cells based on the differential expression of a family of non-coding mitochondrial RNAs (ncmtRNA) which comprises sense (S-ncmtRNA) and antisense (As-ncmtRNA) members. This protocol will be suitable for the detection of tumoral circulating cells as well as descamative cancer cells in urine.
Material and Methods: Double FISH-S was performed in a dozen different cancer cell lines and PHA-stimulated lymphocytes as normal proliferating cells, using ODN probes specific for each one of the ncmtRNAs; the Sense ncmtRNA was identified using a probe labeled with Alexa Fluor 488 (green), and the antisense ncmtRNA with a Texas Red labeled probe. The cells were permeabilized and hybridization was performed adding both probes to the cell suspension and incubated for 15 min at room temperature. A stringency wash of 10 minutes was carried out to eliminate non specific hybridization. The same protocol was performed using voided urine obtained from patients at the Urology Unit of the Hospital Barros Luco Trudeau.
Results: Tumor cell lines of different origins, such as leukemia, melanoma, prostate, bladder, lung, kidney, lymphoma, breast and cervix were easily identified by the FISH-S protocol. Tumor cells showed expression only of the S-ncmtRNA (green). In contrast, normal proliferating cells showed signal with both probes (red and green), indicating a good specificity. The challenge of using voided urine from bladder cancer patients showed that this protocol specifically detected exfoliated tumor cells, indicating that we posess a new and highly versatile cancer diagnostic method.
Conclusions: We developed a short and versatile FISH-S protocol, which can identify a normal or tumor status at the single cell level. The approach, based on the detection of the differential expression of these new cancer biomarkers, can be used for non-invasive detection of circulating or exfoliated tumor cells of bladder and prostate cancer.
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Development of monoclonal antibodies recognizing the active conformation of epidermal growth factor receptor and application for activation-specific measurement in ELISA

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Background: Epidermal growth factor receptor (EGFR) is an important target of anticancer agents, and new anti-EGFR inhibitors and monoclonal antibodies (mAbs) targeting activated EGFR are of continued interest in drug development. We have successfully developed novel mAbs which specifically recognize the active state of EGFR. Our antibodies recognize the epitopes which are exposed through the conformational change induced by ligand-binding but not affected by the subsequent auto-phosphorylation event. Although the phosphorylation state of EGFR is regarded as a surrogate indicator of activated EGFR, phosphorylation merely represents one aspect of EGFR activation. Moreover, since phosphates of phospho-EGFR are highly sensitive to cellular phosphatase during experiments, and EGFR can be phosphorylated via the crosstalk with another kinase without ligand stimulation, use of anti-phospho-EGFR antibodies may not always be the reliable way to detect and quantify activated EGFR. Precise evaluation of activated EGFR using our mAbs would be valuable for drug discovery research and diagnostic applications.
Material and Methods: Mice were immunized with recombinant antigen representing the cytoplasmic domain of EGFR harboring kinase activity. The reactivity of the established mAbs was determined by immunoprecipitation and immunofluorescence. The activated EGFR-specific mAbs were used to establish ELISA. Quantitative measurement of activated EGFR by ELISA was evaluated using lysates prepared from breast and lung cancer cells with or without 100 ng/ml EGF treatment. The lysate of lung cancer cells harboring constitutive active mutation (exon 19 deletion) were also used.
Results: The reactivity and specificity of our mAbs against activated EGFR was confirmed by immunoprecipitation and immunofluorescence. The activated EGFR-specific mAbs immobilized on a plate were found to be able to capture the activated EGFR in cell lysates, indicating that our mAbs are applicable to an ELISA format. We found that our mAbs specifically reacted to the lysates of EGF treated cells and lung cancer cells with constitutive active mutation, while the other mAbs against EGFR reacted to the lysates with or without EGF treatment. We could measure the degree of EGFR activation by EGF stimulation regardless of basal phosphorylation level.
Conclusions: Precise evaluation of activated EGFR was accomplished using the ELISA. The ELISA allows us to measure the quantity of ligand-stimulated EGFR, which are not affected by basal phosphorylation level from crosstalk interaction. Our novel mAbs and ELISA may become novel research and diagnostic tools for detection and measurement of the activated EGFR in various cells and tissues.