K-116151 Genomic analysis of metastatic progression of high incidence cancers: final report

Metastatic lung adenocarcinoma (LADC)

Analysis of 435 metastatic disease demonstrated that metastasis to the lung, pleura and adrenal glands occurred significantly earlier, whereas brain metastases appeared significantly later in lung cancer patients. By clustering analysis, we found that brain metastases tend to appear together with bone and lung metastases. Central primary tumors were significantly associated with early (and frequently bone-) metastases whereas lung metastases associated with peripheral tumors. Central primary LADCs were associated with significantly decreased median overall survival both in univariate and in multivariate analyses. (**Fábián K et al. 2016)** Check point inhibitors are main immunotherapy targets in lung cancer frequently using PD1/PDL1 as biomarkers. We have analysed 208 brain metastatic lung cancer cases for mononuclear cell infiltration and PD1/PDL1 expression. Our data indicated that high density of infiltrating mononuclear cells and low expression of PD1/PDL1 in metastases are associated with prolonged overall survival suggesting an active antitumoral immune response even in brain metastasis. (**Téglási et al. 2017**)

Using a large lung adenocarcinoma cohort of 268 cases (131 test-cohort and 137 validation cohort) the checkpoint inhibitori ligand PDL1 and its receptor PD1 expressions were analysed on tumor cells (TC) and tumor infiltrating lymphoid cells (IC) in correlation with the genetic status of the tumor, EGFR- or KRAS mutations. PDL1 expression on TC was positively correlated with the ligand expression on IC. One cause for PDL1 expression of TC is necrosis in the tumor tissue, since the presence of this resulted in a significantly higher ligand expression. Interestingly, tumor necrosis resulted in a higher PD1 receptor expression on IC cells suggesting that this phenomenon is a major factor in negatively regulating antitumoral immune responses. EGFR mutation status is negatively correlated with PD1 receptor expression on TC and IC but did not correlate with PDL1 ligand expressions on TC or IC. Similarly, KRAS mutation status did not affect PDL1 or PD1 protein expression on TC neither on IC. This later result contradicts some earlier reports suggesting a correlation between PDL1 expression and KRAS mutation status of the lung adenocarcinoma. (**Téglási 2019**)

An analysis of a large clinical cohort of metastatic lung adenocarcinoma (1126 pts) revealed that central tumors metastize earlier than peripheral ones. It was also detected that bone metastases are more frequent in case of central primary tumors while in case of peripheral ones lung metastases are more frequent than any other type of metastases. (**Klikovits 2018**) Immune checkpoint inhibitor therapy is now a standard in lung cancer therefore PDL1/PD1 expression is a key issue. In a small non-small cell lung cancer cohort which was pretreated with chemotherapy the expression of PDL1 protein was assessed on tumor cells and immune cells of the primary tumors. Our data indicated that cisplatin/gemcitabine therapy caused a decreased PDL1 expression on tumor cells unlike the carboplatin/paclitaxel combination suggesting that gemcitabine may be responsible for such a downregulation. (**Rojkó 2018**) In a cohort of 61 brain metastatic lung adenocarcinoma we have compared the PD1/PDL1 protein expression of metastases as compared to the primary tumor. During the metastatization chemo- and radiotherapy were performed. It was observed that the PDL1 protein expression in brain metastases was similar to the primary tumor on tumor cells and was not affected by the therapy introduced. However there was a large difference in immune microenvironment of the brain metastases as compared to the primary tumor which was also reflected by PD1/PDL1 expression. (**Reiniger 2019**)

The Central East-European Lung Cancer working group run a comprehesive survey on the molecular testing of lung adenocarcinoma in the region. From Hungary 6 centers participated in the survey on EGFR and ALK mutation testing. Data clearly indicated a large discrepancy of revealing EGFR- or ALK-mutant lung cancers in the various Hungarian centers: EGFR mutation rates 6.8-14.9%, ALK mutation rates 1.6-8.5%. This is the first report on the quality of molecular testing of lung cancers in Hungary suggesting major efficiency differences between the testing centers. (**Ryska et al.2018**)

Our earlier experimental work demonstrated that the efficacy of prenylation inhibitor treatment of human lung adenocarcinoma cells with Zometa/aminobisphosphonate is dependent on KRAS wild type status. (**Kenessey et al. 2016**) Accordingly, we tested this in clinical situation. KRAS mutation is the most common genetic alteration in lung adenocarcinoma (LADC) in Western countries and is associated with worse outcome in bone-metastatic cases. Yet, to date, no treatment guidelines were developed for these patients, although bisphosphonates are registered for bone metastatic cancers. Accordingly, our aim was to investigate the impact of KRAS mutation on bisphosphonate (BTx) and radiation therapy (RTx) in bone-metastatic LADC patients. Clinicopathological variables of 134 consecutive LADC patients with bone metastases at diagnosis and known KRAS status were retrospectively analyzed. The effects of BTx, RTx and KRAS mutation on overall survival (OS) were investigated. Of the total cohort, 93 patients were identified as KRAS wild-type (WT) (69.4%) and 41 (30.6%) as KRAS-mutant patients. The presence of KRAS mutation was associated with significantly reduced median OS (5.1 months, vs. 10.2 months in KRAS-WT patients; p=0.008). Irrespective of KRAS mutational status both BTx (p=0.007) and RTx (p=0.021) conferred a significant benefit for OS. Notably, however, when analyzing the patients with KRAS-mutant and KRAS-WT tumors separately, the benefit from BTx and RTx on OS remained statistically significant only in KRAS WT patients (p=0.032 and p=0.031, respectively). KRAS mutation is a strong negative prognostic factor in bone-metastatic LADC patients. (**Radeczky P et al. 2021**) Altogether, KRAS mutational status should be considered during therapeutic decision making in bone-metastatic LADC patients. Since KRAS mutation to date is considered only as an EGFR-TKI resistance marker, its routine determination must be extended. (**Tímár J et al. 2020**)

Metastatic breast cancer

124 metastatic breast cancer data have been evaluated to analyse metastatic patterns of the molecular subgroups. The longest distant metastasis-free survival (DMFS) characterized the highly differenciated Luminal-A (ER/PR+/HER2-) subtype, while the shortes DMFS occured in the HER2 amplified group. The majority of the Luminal-A tumors (59%) formed solitary metastasis primarily to bone, while the majority of HER2+ tumors formed multiple metastases (79.2%). The second most frequent metastatic sites in Luminal-A tumors were lung and liver. In case of HER2+ or triple negative breast cancers brain was shown to be the most frequent metastatic organ. These data strongly suggest that the metastatic potential of breast cancer is driven by the inherent basic molecular characteristics, expresion of hormone receptors and HER2 gene amplification. (**Molnar et al. 2017**)

Homologous recombination deficiency (HRD) is the result of genetic alterations of cancers including BRCA mutations. With the advent of PARP inhibitor therapy it is possible to molecularly taget HRD deficient tumors including breast- or ovarian cancers. We have studied the incidence of HRD deficiency in a public dataset of 21 breast cancer brain metastases and corresponding primary tumors using various algorithms: HRD-LOH, large-scale state transitions (LST) and telomeric allelic imballance assay (mtAI) and the combination of them

called as MyChoice (Myrad Genetics). A fourth analysis tool of HRDetect was also included. Data indicated that HRD scores measured by either methods significantly increased in brain metastases of breast cancers. For validation analysis we have used a cohort of 16 brain metastatic cases and run a WES sequencing on primary and metastatic FFPE tumors. MyChoice analysis indicated that 14/16 cases brain metastases showed a significantly increased HRD scores as compared to the primary. Even more important is the observation that in 4 cases brain metastases switched the HRD status from negative in primary to positive (based on the accepted thresholds). On the other hand, in two BRCA-mutant tumors HRD positivity was maintained in brain metastases. (**Diossy et al.2018**) These observations are very important since indicate a genome-wide development of aberrations during brain metastatisation of breast cancers. The question remains that this development is organ specific or metastasis specific. To study this, lung- or liver metastatic cases will be re-analysed by WES and MyChoice; such cohorts are available at our Department. Learned from our experience on HRD in brain metastases of breaat cance, we have systematically evaluated the literature data on genomic alterations during tumor progression from the point of view of immuno-oncology. We found evidences that tumor mutational burden is not stable during tumor progression and mostly is increasing due to various forms of DRDs caused by novel alterations in DNA repair enzymes. However, one particular cause, the APOBEC activity does not lead to increased sensitivity to immune attacs. HLA loss or PDL1 amplifications are also significant factors of immunoresistance or sensitivity, and these genetic alterations may occure at any point of metastatic progression. It is also important to realize that even the primary tumors are heterogenous clonally from the point of immunogenomic aberrations, and this is mainly the source of alterations detectable in the metastaic tissues. Accordingly, such main immunogenomic characteristics must be re-tested in metastatic tissues before making therapeutic decisions. (**Ladányi et al. 2020**)

Bone metastatic clear cell renal cancer (cRCC)

59 non-metastatic and 40 bone metastatic cRCC tumors were analysed for HIF1a/HIF2a mRNA and protein expressions as well as for their 7 target genes. We have found that high HIF1a and low HIF2a expressions are prognostic markers for bone-metastatic disease associated with shorter survival. Furthermore, high HIF2a is associated with high VEGFR2 as well. These data have been confirmed in 399 bone metastatic cRCC cases of TCGA database. (**Szendrői et al. 2016)**

Metastatic malignant melanoma

We performed a genome-wide copy number variation (CNV) analysis on 12 primary and their 34 visceral metastatic samples using OncoScan- (on fresh frozen samples) or Cytoscan assay (formalin-fixed paraffin embedded samples). The 12 cases included 5 BRAF-, 4 double wt and 2 NRAS mutant cases. When data were aggregated according to the mutation status and the localization of melanoma metastases, lung metastases were excluded from further analysis, because all lung tumors were BRAF mutant. Brain^{BRAF} represented specific copy number gains on only 8q, including important "historical" genes of DNA damage repair machinery (*PRKDC, RRM2B, UBE2V2, UBE2W*) and a key player of cell survival regulation, *YWHAZ*. Another gene should be mentioned here, *TERF1* is proven to be important in telomere maintenance and melanoma development as well. *NRAS* mutation bearing brain metastases were enriched exclusively in gains of chr2, 8p and 22q localized genes, such as FGF9/20 subfamily signaling. FGF9 regulates vascular development. Members (*ITGA4,-A6,-AV*) of the integrin gene family were also affected by copy number gains; also important indicators of tumor stromal influence

on tumor cells in the same angiogenesis related manner. Genes altered by LOH in liver^{BRAF} metastases in chr2, chr 8p and 8q24.1 and other additional alterations on 17q, 20q were detected, too. Beside several histon genes, HLA genes are located in these regions. In liver^{NRAS} samples CNA enriched pathways were quite similar to the ones described above in connection with *NRAS* mutant without distinguishing between different metastatic sites. Plenty of drug metabolism related and IL-1 pathway-related genes had CNA gained. Exclusively, compared to *BRAF* mutant liver metastases, TNF signaling (e.g *TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, CARD14, BAG4, IKBKB*) was also affected by copy number gains. Liver^{WT} metastases were different from others in their LOH landscape, regarding LOH of genes (e.g *IL-13, -9, -4, -3*) in 3q11.2, 5q31.1, 7q11.22 and 11p11.12 only.

Liver metastatic colorectal cancer

We have collected a relatively large colorectal cancer cohort of 97 cases of primary tumors and 41 cases of corresponding metastases 58% of which was in the liver. In another collaborating German center we have a large cohort of colorectal cancer lung metastases with corresponding primary tumors. As a third important site a significant 45 case cohort of colorectal cancer brain metastases with corresponding primary tumors are avaiable at a Budapest Hospital.

There are several controversial reports on the association between EGFR copy number (CNV) and EGFR protein expression in colorectal cancer, the key target of anti-EGFR antobody therapy. In our primary and metastastatic tumors we have analysed this association and found that EGFR CNV does not affects EGFR protein expression using a semi-quantitative H-scoring evaluation for protein and an absolute measurement of EGFR copies by FISH. While EGFR CNV was in the range of 1.9 (diploid) and 5.04 (amplified) the EGFR protein H-scores were in the range of 5-250 without any association. (**Uhlyarik 2019**)

It is a novel clinical observation that left sided colorectal cancer has a poorer prognosis than right-sided one and also that left-sided colorectal cancers respond better to anti-EGFR therapies. In a cohort of 97 KRAS-wt primary colorectal cancers we have evaluated EGFR protein expressions along the entire colorectum. We have observed that the highest EGFR protein expression as defined by H-score was detected in the coecal and ascending colon tumors as compared to a significantly lower EGFR scores in the distal transverse colon down to descending colon, sigma and rectal tumors. Comparison of the left- and right-sided colorectal cancers EGFR expressions, we found a significantly lower protein expression in the left-sided tumors. Since all of these patients have been treated with anti-EGFR antibody therapy, we have compared the PFS and OS of the left- and right sided tumor-carrying patients. We have seen, as it is reported, that anti-EGFR antibody therapy efficacy is significantly better in case of the left-sided cancer cases. In this cohort we found a negative correlation between EGFR protein expression and the efficacy of the anti-EGFR antibody therapy of KRASwt-colorectal cancers.

The positive predictive marker in colorectal cancer for anti-EGFR antibody therapy is missing, therefore we use RAS-mutant status as negative predictor for patient selection. We have analysed 99 primary and 32 RAS-wild type liver metastatic colorectal cancers which were treated with anti-EGFR antibody therapy. We have analysed EGFR protein expression in primary and liver metastatic tumor tissue as determined by an ivd. methodology and patient survival was analysed for efficacy. We have found by surprise, that in primary tumor low or negative EGFR expression is the best (though not significant) positive predictor. In liver metastases using several thresholds it became evident that the low levels of EGFR protein on tumor cells serves as positive predictor of response and survival. (**Uhlyarik 2020**) Re-

evaluation of the entire cohort revealed that beside tumor cells there are several cases where tumor stroma expressing EGFR protein and this expression is frequently accompanied by low or no expression of EGFR on tumor cells. Identification of the EGFR positive stromal cells and evaluation of their predictive role is ongoing. To analyse further this and to reveal novel predictors for the efficacy of anti-EGFR therapy, we have collected a 27 pt cohort of anti-EGFR treated KRAS wt colorectal cancer patients where both the primary tumor as well as liver metastases are available for genomic analysis. The samples are now prepared and submitted to sequencing using Oncomine target panel of 150 genes using IonTorent instrument. Raw data are obtained, bioinformatics analysis is ongoing.

We have demonstrated that KRAS mutant lung adenocarcinoma is resistant in vitro and in patients to prenylation inhibitory therapy, aminobisphosphonate (Zometa). Therefore, we have run a short in vitro analysis in human colorectal cancer cell lines of double wt (KRAS/BRAF), KRAS mutant and BRAF mutants for sensitivity to prenylation inhibition using Zometa and a novel derivative. By surprise, we have observed that prenylation inhibition can have antiproliferative effects in KRAS-mutant colorectal cancer cells suggesting that sensitivity to molecularly targeted therapies is tumor-type specific. This situation seems to be similar what is known for BRAF mutant melanoma and colorectal cancer, where melanoma is sensitive while colorectal cancer is resistant to BRAF inhibitors. (**Baranyi et al. 2020**)

BRAF mutation in colorectal cancer is a strong negative prognostic factor and a negative predictor in respect of anti-EGFR therapy, accordingly its determination is now part of the routine diagnostics. However, and unlike in melanoma, mutant BRAF inhibitors are not effective in colorectal cancer due to feedback EGFR-activation mechanisms. We have modelled this situation in vitro where we have collected BRAF mutant and BRAF and PI3K/PTEN double mutant cell lines and analysed the effect of MEK inhibitor alone or in combination with PI3K/mTOR inhibitor. Data indicated that MEK inhibitor is more effective (effective!) in BRAF mutant only cell lines. However, MEK inhibitor in combination with PI3K/mTOR inhibitor became effective in so-called double mutant cell lines (BRAF + PI3K/PTEN). These data suggest that determination of the the PI3K/PTEN mutation status of colorectal cancer can be necessary to design effective target therapies for BRAF mutant colorectal cancer. (**Rittler et al. 2020)**

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