IMADETECT™: Mass spectrometry guided development and clinical application of a companion diagnostic for adoptive cellular therapy against tumor associated HLA peptides

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Background

Adoptive cellular therapy (ACT) has demonstrated substantial clinical progresses in hematologic cancers; however, only a small proportion of solid tumor patients have benefited from these advances. Two ACT approaches currently tested in phase I are addressing this need: ACTolog® using autologous T-cell products against multiple targets and ACTengine® using T cells engineered to express cancer specific T-cell receptors. Both approaches rely on proper identification of patients that are positive for at least one of the targets. The method of choice in a clinical setting is the quantitation of mRNA expression by quantitative polymerase chain reaction (qPCR). Yet, since the actual target is a peptide bound to human leukocyte antigen (HLA), correlation between gene expression and peptide presentation has to be confirmed. While global correlation between transcriptome and HLA peptidome has been described [1] , individual correlation on peptide-level is disconnected [2]. In a second step an appropriate qPCR threshold has to be defined allowing prediction of peptide presentation. Here we describe how HLA-A*02 positive tumor samples analyzed by the XPRESIDENT® target discovery engine are used to translate measurements by liquid chromatography – mass spectrometry (LC-MS) to RNA-sequencing (RNAseq) and further to qPCR [3]. We describe how the clinical assay was established and validated and present first screening results from the ACTolog® IMA101 trial.

Correlation between transcriptome and HLA ligandome

To investigate the correlation of an individual HLA peptide with its corresponding gene expression, we analyzed 170 HLA-A*02 positive tumor samples with paired LC-MS and RNAseq measurements taken from the XPRESIDENT® database (Fig. 1). One observation was that only a few peptides show a strong correlation between peptide abundance and corresponding gene expression (Fig. 2A/B). Second, correlation as metric is restricted to samples with pair-wise complete data. Due to sparseness of LC-MS data, this might ignore a considerable amount of data points (Fig 2C).

Figure 1. Overview of the XPRESIDENT® platform. HLA-bound, tumor-associated peptides (TUMAPs) are identified and quantified directly from primary human tumor and normal tissue samples by liquid chromatography tandem mass spectrometry (LC-MS/MS). Over-presentation on peptide level is corroborated by RNA over-expression using RNAseq. Furthermore, healthy tissue analyses help to predict potential on- and off-target toxicities.

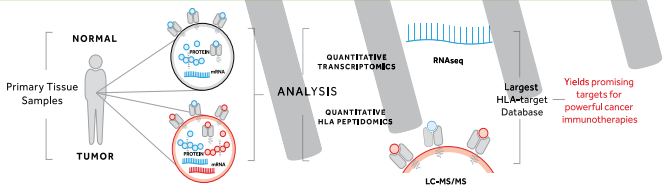
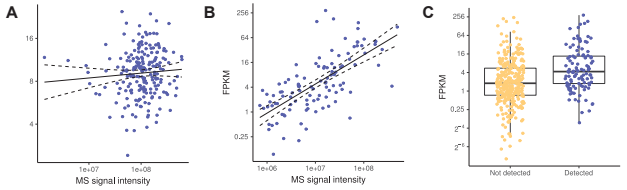


Figure 2. Distorted relationship between HLA peptidome and transcriptome. Correlation for (A) peptide TSC2p526 (R=0.07) and (B) phosphorylated SYNMp426 (R=0.68) between the peptide's LC-MS signal intensity (XIC, extracted ion chromatogram) and gene expression (FPRM, fragments per kilobase of exon per million reads mapped). (C) Only for 1 out of 4 samples an LC-MS signal was detected for SYNMp426. Thus only for this subset the correlation can be investigated (Fig. 2B). High correlation therefore does not necessarily indicate that a gene expression threshold will be predictive since samples without peptide detection might show high gene expression.



Mass spectrometry-guided mRNA threshold

For target and patient selection in immunotherapies targeting peptide-HLA complexes, it is necessary to establish an mRNA threshold predictive for presentation of the particular HLA-peptide. This was accomplished in a two-step approach. First, peptide presentation was modelled by RNAseq to translate from peptidome to transcriptome using the XPRESIDENT® data. The model adjusted for the depth of the underlying peptidome analysis (peptidome measurement depth, PMD) by considering the number of identified HLA-A*02+ peptides (Fig. 3A). The RNAseq threshold was determined by maximizing the F-score for prediction of samples with peptide detection. This optimization took into account that detection of a peptide at low PMD (shaded area) is less likely. This PMD threshold depends on the lower limit of detection of the peptide and was also estimated as part of the optimization. In a second step, the RNAseq measurements were mapped to qPCR measurements using a calibration curve (Fig. 3B). The calibration curve was acquired using a validated qPCR assay described below.

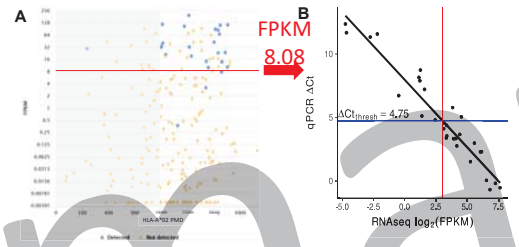
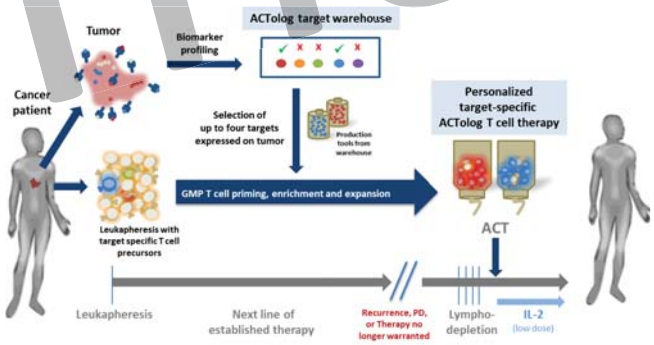


Figure 3. Determination of mRNA thresholds guided by mass-spectrometry for an exemplary ACTolog target.

(A) Detetction by LC-MS is predicted by RNAseq correcting for PMD.

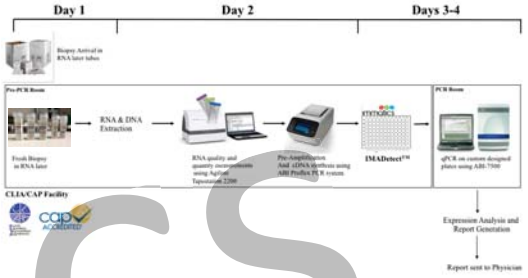
(B) Calibration curve between RNAseq and qPCR mRNA expression measurments.

ACTolog®: Study Design



ACTolog® is a first-in-human clinical trial in patients with relapsed or refractory solid cancers. If the patient is HLA-A*02:01 positive and all other initial eligibility criteria are met, a tumor biopsy will then be performed and tested by IMADETECT™ as investigational diagnostic assay using qPCR. The collected tumor material will be tested for the expression of warehouse target(s). The qPCR thresholds at which a target is considered positive are guided by mass-spectrometry as described above. Patients will undergo leukapheresis to collect mononuclear cells for manufacturing of IMA101 T-cell products. IMA101 will be infused after pre-conditioning regimen (lymphodepletion) followed by low-dose IL-2. The study plans to treat 20 patients with ACTolog® IMA101 products.

qPCR assay



IMADetect™ is an investigational non-invasive *in vitro* companion diagnostic device (IVD) to be used and co-developed together with the investigational new drug IMA101. The intended use of the device is to provide data enabling a physician to decide against which of the eight cancer targets specific T cells should be manufactured and adoptively transferred to treat a patient presenting with a diagnosed cancer disease. The result of the device determines if none, one or more targets could be addressed by the T-cell therapy. IMADetect™ requires a small piece of the patients' cancer biopsy, which is then analyzed for target gene expression using qPCR (processing time 3-5 days). IMADetect™ is used in a CLIA/CAP-certified laboratory.

Patient screening and target selection

Patient	Indication	AG003-02	AG018-01	AG008-01	AG012-01	AG016-02	AG001-02	AG007-01	AG013-01
1	Synovial sarcoma	+	-	+	+	-	-	+	-
2	Breast cancer	-	-	-	+	+	-	-	-
3	Ovarian cancer	-	-	+	+	+	-	-	+
4	Myxoid liposarcoma	-	+	+	+	-	-	-	-
5	Synovial sarcoma	-	+	+	+	+	-	-	-
6	Met. clear cell sarcoma	-	-	-	+	-	-	-	+
7	Pancreatic cancer	-	-	-	+	-	-	-	-
8	Endometrial carcinoma	-	-	-	+	-	-	-	-
9	HNSCC	-	-	-	+	-	-	-	+
10	Breast cancer	-	-	+	+	-	-	-	+
11	Esophageal cancer	-	-	+	+	-	+	-	-
12	Nasopharyngeal cancer	-	-	-	+	-	-	-	+
13	Cervical cancer	-	-	-	+	-	-	-	-
14	Metastatic adenoid cystic carcinoma of Bartollins gland	-	-	-	-	-	-	-	-

Acknowledgement & Future Development

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- The enrollment is ongoing.

References

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