SUMMARY/ABSTRACT

In this study attempts were made to: i) collect and collate data from various resources to develop a comprehensive database on Herceptin (anti-HER-2 monoclonal antibody) resistance, ii) characterize anti-EGFR monoclonal antibody (MAb), taken alone or in combination with other drugs, for its cytotoxic effect on various cancer lines and, iii) characterize anti-TNF-α MAb and its reactive mimotope.

A systematic attempt was made, under collaborative work with Dr. G.P.S. Raghava's group at IMTECH, to collect and compile data from various resources to develop a comprehensive database on Herceptin Resistance (HerceptinR). This database contains information about 2500 assays, 30 cell lines and 100 supplementary drugs. Numerous user-friendly tools have been integrated, for searching, browsing and alignment of the data pertinent to Herceptin resistance. It also provides detailed information useful in Herceptin treatment. For instance, Herceptin assay data module mentions the experimental details, such as antibody (Ab) amount, time of Ab treatment (*in vitro*), supplementary drug, drug amount, time of drug treatment (*in vitro*), percent inhibition, experimental techniques followed and testing Herceptin resistance with different cell lines having defined alterations. Besides, this study demonstrates the utility of the database in retrieving the information which may be useful in designing personalized treatment of a patient. Some of the applications of HerceptinR demonstrated in this study included the retrieval of information about: i) available Herceptin sensitive and resistant (*de novo* /acquired) cell lines, ii) Herceptin response in resistant (*de novo*) and sensitive breast cancer cell lines, iii) combinatorial response of Herceptin and drugs in Herceptin sensitive and *de novo* resistant breast cancer cell lines and, iv) combinatorial response of Herceptin and drugs in acquired resistance for Herceptin in breast cancer cell lines. Thus, HerceptinR database will be useful in providing vital information while designing Herceptin based treatment.

Mouse MAb D1G4, generated in the lab against A431cells, exhibited good reactivity with purified human EGFR in dot blot assay. It also reacted well with A431 cell surface as revealed by FACS analysis and confocal microscopy. Importantly, this MAb also showed antiproliferative activity against A431 cancer cells. Combinatorial treatments of necitumumab (anti-EGFR) and

trastuzumab (anti-HER-2) showed differential effects on various cell lines: i) in A431 cells, necitumumab inhibited the growth of the cells, while trastuzumab had no effect and in combination it partially inhibited the necitumumab inhibitory effect on cell growth, ii) in A549 cells, like A431 cells, both necitumumab and trastuzumab exhibited similar effects. However, both antibodies, in combination, significantly inhibited the growth of these cells, iii) in SK-BR-3 cells, unlike necitumumab, trastuzumab was growth inhibitory, while like in A549, synergistic growth inhibitory effects of both the antibodies were also observed in this cell line and, iv) in MCF-7, the inhibitory effects of both necitumumab and trastuzumab, taken alone, was quite low (15-25%), while in combination the observed inhibition of cell growth was around 50%.

Combinatorial treatments of trastuzumab, with various target specific inhibitors, were checked taking SK-BR-3 (HER-2 over expressed) and MCF-7 (with low HER2 expression) cell lines. Results showed: i) synergistic effects between bosutinib (src inhibitor) and trastuzumab in HER-2 over expressing SK-BR-3 and MCF-7 cell lines, ii) synergistic effect of PF-04691502 (PI3k-mTOR dual inhibitor) in SK-BR-3 and, iii) no synergism of 17-AAG (HSP90 inhibitor) with trastuzumab in MCF-7 but potent effect in SK-BR-3. Thus, these results demonstrated that different drugs or inhibitors may behave differently, in synergism with MAb, depending upon the surface architecture of the cancer cell.

MAb 9G9, generated in the lab, showed good reactivity with purified human TNF-α in dot blot assay while BIACORE analysis of 9G9 binding with TNF-α showed that this antibody has much lower affinity as compared to that of human MAb adalimumab (Humira). Nevertheless, this MAb did exhibit the TNF-α neutralization property when checked in L929 based assay. Interestingly, in sandwich ELISA, 9G9 detected human TNF-α in a range between 1 ng/ml to 10 ng/ml level. Thus, it would be of interest to test this MAb for the detection of TNF-α in biological samples.

Clustal X alignment of 7-mer cysteine constrained phage peptide (T6), isolated by panning phage peptide library on commercial mouse anti-TNF-α MAb (Santa Cruz), showed its resemblance with aa 141-148 functional region of human TNF-α. This phage peptide, upon immunization, was able to induce parent antibody type response. ELISA and western blotting,

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using this phage peptide, showed that the epitope of 9G9 may be similar or overlapping with that recognised by commercial mouse MAb, but differed from that reactive with Humira. Further, the synthetic peptide corresponding to T6 reacted well with both the mouse MAbs, but not with Humira, in sandwich ELISA. This further confirmed that 9G9 and Humira are directed towards different epitopes. It is likely that this peptide may find its application for designing TNF-α detection assay and also for generating neutralizing anti- TNF-α antibodies.

In nut shell, the lab generated anti-EGFR and anti- TNF-α monoclonal antibodies and peptide mimotope of TNF-α neutralizing MAb, described in this study, may be exploited in development of human EGFR and TNF-α based therapeutics and/or diagnostics. Also, synergistic effects of Herceptin, with drug or anti-EGFR MAb, observed in this study may have therapeutic implication on Herceptin treatrment in Herceptin resistant patients.