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Upcoming techniques in drug development

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ABSTRACT: Under development are new techniques that have the potential to define subpopulations of patients which may or may not respond to targeted therapy. Better definition of resistance mechanisms against monoclonal antibodies or tyrosine kinase inhibitors will be one approach to achieve this goal. Progress has been made in the detection of these mechanisms when targeting the epidermal growth factor (EGF) receptor family. Proteomics either performed by imaging techniques or by methods such as matrix-assisted desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry requires further standardisation and validation. A mass spectroscopy-based pre-treatment patient selection system is under development that is highly reproducible and capable of classifying patients by survival.

Keywords: Epidermal growth factor receptor; Resistance mechanisms; Proteomics; Imaging MALDI-TOF; Mass spectrometry

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DEFINITION OF RESISTANCE MECHANISMS

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Only a fraction of patients respond initially to therapy (primary resistance), and those who do, often lose their response to targeted therapy with time (secondary resistance). The postulated mechanisms behind targeted therapy and development of resistance were explored and the epidermal growth factor receptor (EGFR) family was used as an example. The ErbB or epidermal growth factor (EGF) family of receptor tyrosine kinases consists of four members: EGFR (Erb1/HER-1), HER-2 (ErbB1/neu), HER-3 (ErbB3), and HER-4 (ErbB4). ErbB receptor tyrosine kinases are attractive targets for therapy. They initiate multiple signalling pathways, and they are conveniently accessible on the cell surface where they can be targeted with monoclonal antibodies.[1] They have an ATP-binding site and are amenable to enzymatic blockade. Mutations - often single amino acid replacements and short deletions – in *ErbB* receptor genes can occur, as is the case with non-small-cell lung cancer (NSCLC). Likewise, overexpression of ErbB proteins occurs in a fraction of carcinomas, including ErbB-2 overexpression in about a third of breast cancers. Cetuximab (Erbitux®), panitumumab (Vectibix®) and trastuzumab (Herceptin®) are all examples of anti-ErbB monoclonal antibodies in clinical use. The potential mechanisms of trastuzumab action include

- recruitment of natural killer (NK) cells to Her-2 overexpressing tumour cells;
- reduction of Her-2 signalling to the phosphatidylinositol-3 kinase (PI3K), Src, or mTOR pathways;
- inhibition of angiogenesis and vasculature normalisation, thereby improving delivery of chemotherapeutic drugs such as paclitaxel (Taxol®);
- enhancing receptor endocytosis and degradation;
- other hypothesised mechanisms.

Perhaps the main mechanism of action is antibody-dependent cellular cytotoxicity (ADCC) of tumour cells. Antibodies attach to the tumour cell, then NK cells move in and destroy it. Clynes compared knockout and wild-type mice and showed that preferential activation of inhibitory Fc receptors enhanced tumour responses to trastuzumab treatment.[2] Mice without Fc receptors were unable to recruit NK cells, hence the antibody displayed only partial inhibition of tumour growth.

Spiridon and colleagues used mice with severe combined immune deficiency (SCID) to demonstrate synergistic antitumour effect of a combination of monoclonal antibodies against Her-2 that do not bind to the same epitope.[3] The speaker presented studies performed in his laboratory, in collaboration with M. Sela, showing that single monoclonal antibodies lead to some down-regulation and degradation of EGFR, but certain combinations completely destroy the receptor. A single monoclonal antibody leads only to dimer formation, yielding inefficient

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internalisation, whereas multiple monoclonal antibodies lead to efficient internationalisation by formation of an antigen–antibody lattice at the cell surface.

An example was shown that involved combining two monoclonal antibodies, trastuzumab and pertuzumab, which do not compete for the same HER-2 site. The combination was much more effective than either used alone.

DEVELOPMENT OF RESISTANCE TO ANTI-ERBB RECEPTOR ANTIBODIES: Only a third of breast cancer patients with Her-2 overexpressing tumours respond initially to trastuzumab, implying that most patients have primary resistance. Also the majority of responding patients demonstrate disease progression after months of treatment initiation because they develop secondary resistance. Nagata et al. described the development of resistance to monoclonal antibodies via deficiency of PTEN, a dual phosphatase that mainly dephosphorylates position D3 of membrane phosphatidylinositol-3,4,5 trisphosphate. PTEN-deficient breast cancer patients had significantly poorer responses to trastuzumab-based therapy than those with normal PTEN levels. The authors concluded that PTEN deficiency predicted resistance to trastuzumab and that PI3K inhibitors rescued PTEN loss-induced trastuzumab resistance in a model system, suggesting PI3K-targeting therapies as a means of overcoming acquired resistance.[4]

Another possible mechanism for resistance to monoclonal antibodies against tyrosine kinase receptors was described by Nagy et al. who claimed that MUC4, a membrane-associated glycoprotein that inhibits trastuzumab binding to Her-2, was higher in a trastuzumab-resistant cell line than in sensitive cell lines.[5] Levels of MUC4 were inversely correlated with the trastuzumab-binding capacity of single cells. Knockdown of MUC4 expression by RNA interference increased binding. It is postulated that masking of ErbB2 by MUC4 is a mechanism for trastuzumab resistance. In addition resistance may occur via insulin-like growth factor-I (IGF-I) receptor interactions, as described by Nahta and colleagues.[6] They found that Her-2 interacts in a unique way with IGF-I receptor in trastuzumab-resistant cells possibly enabling cross talk between IGF-I receptor and Her-2. The IGF-I receptor/Her-2 heterodimer could contribute to trastuzumab resistance and serve as a potential therapeutic target in breast cancer patients whose disease has progressed while on trastuzumab.[5]

In conclusion, defective immune responses (e.g. Fc receptors) might underlie primary resistance to trastuzumab, but acquired (secondary) resistance seems to involve compensatory signalling mechanisms.

Various reports have addressed the issue of resistance to tyrosine kinase inhibitors (TKI). Despite significant responses to gefitinib (Iressa®), most patients with non-small-cell lung cancer (NSCLC) relapse after 6–18 months. Kobayashi et al. described the case of a patient with *EGFR*-mutant, gefitinib-responsive, advanced NSCLC who relapsed after 2 years of complete remission. Secondary resistance developed via a second point mutation in EGFR.[7] This phenomenon might be responsible for germ-line mutations in families with multiple cases of NSCLC. It is not known whether this mutation exists independent of gefitinib exposure. Acquired resistance to lapatinib was associated with up-regulation of oestrogen receptor signalling.[8] These findings appear to provide a rationale for preventing the development of acquired resistance by simultaneously inhibiting both oestrogen receptor and ErbB2 signalling pathways.

In summary tumours demonstrate remarkable plasticity, seeming to switch pathways when one is effectively inhibited. Clarifying the mechanisms of drug action is critical for understanding resistance to targeted therapies. Resistance mechanisms richly harness compensatory pathways (PI3K/Akt, Her-3 and IGF-I receptor). Understanding drug resistance will likely identify novel drug combinations and elucidate the mechanisms of tumour cell escape, which must involve the ability to up-regulate certain pathways.

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APPLICATION OF NOVEL IMAGING TECHNIQUES FOR EARLY CLINICAL TRIALS

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