Axel Ullrich

MOLECULAR MEDICINE 1977-2013

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Axel Ullrich

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1977-2013

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Molecular medicine in the last 45 years means breakthrough research in genomics, proteomics, bioinformatics, antibodies, small molecular drug discovery, high throughput screening, signal transduction therapy, targeted therapy, personalized therapy and several other related research areas. But the whole story started with basic discoveries in genomics and molecular biology where I was lucky enough to be involved from the very early stage.

In the 1970s and 1980s, at a very early stage of gene technology development, with my colleagues at UCSF and Genentech we worked out the Method for Producing Recombinant Bacterial Plasmids Containing the Coding Sequences of Higher Organisms and we were the first to clone genes of medically important human proteins such as insulin which later in 1985 led to the first gene technology-based therapeutic agent – Humulin (Lilly). At UCSF in 1977 we constructed the first plasmids containing the coding sequences of the rat insulin genes[1]. Based on this research already at Genentech I determined the Nucleotide sequence of human pre-proinsulin complementary DNA in 1980, which led to the development of Humulin[2]. We created and patented the recombinant bacterial plasmids containing the coding sequence of insulin genes.

This research logically led to attempts towards the molecular genetic characterization of small growth factors like EGF and corresponding cell surface

[1] Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W.J., and Goodman, H.M. (1977) Rat insulin genes: Construction of plasmids containing the coding sequences. *Science* 196 (4296), 1313-1319.

[2] Sures, I., Goeddel, D., Gray, A., and Ullrich, A. (1980) Nucleotide sequence of human preproinsulin complementary DNA. *Science* 208 (4439), 57-59.

receptors. The cloning of cDNA encoding the epidermal growth factor receptor (EGFR), what we published in Nature in 1983[3], opened up a research field that is known today as "Signal Transduction" research.

The breakthrough discovery of Bishop and Varmus that cancer inducing genes of animal retroviruses such as v-src or v-ras represent mutated host genes that were recombined into the viral genome raised the question of whether the oncogene concept was also relevant to human cancer. The first cloning and sequence analysis of a cDNA encoding a cell surface protein, the human EGF receptor, provided a partial answer to this question by revealing a close relationship with the v-erb-B oncogene[4,5].

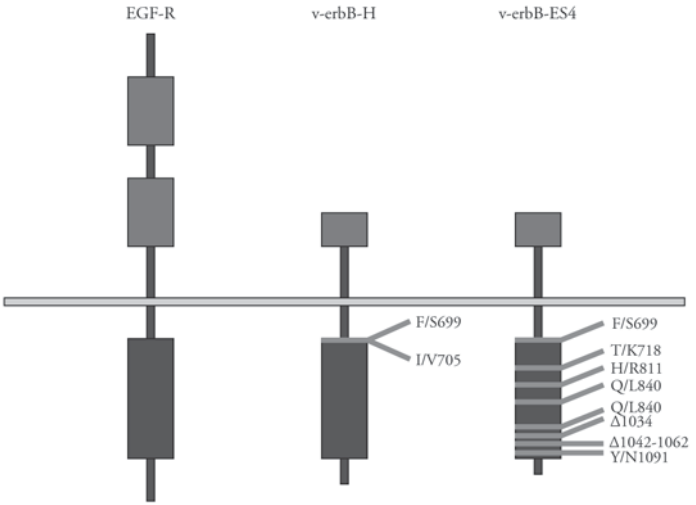

Fig 1: Similarities between EGFR and v-er-B

[3] Gray, A., Dull, T.J., and Ullrich, A. (1983) Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000-molecular weight protein precursor. *Nature* 303 (5919), 722-725.

[4] Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J., and Waterfield, M.D. (1984) Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307, 521-527.

[5] Ullrich, A., Coussens, L., Hayflick, J.S., Dull, T.J., Gray, A., Tam, A.W., Lee, J., Yarden, Y., Libermann, T.A., Schlessinger, J., Downward, J., Mayes, E.L.V., Whittle, N., Waterfield, M.D., and Seeburg, P.H. (1984) Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309 (5967), 418-425.

This first connection between a human gene product that regulates normal cell proliferation and a viral oncogene strongly suggested that human cancer development may also involve abnormalities in the expression and structure of endogenous genes that have regulatory roles in cell proliferation. A search for such genetic aberrations in tumor tissues using cDNA probes of EGFR and an accidentally cloned EGFR related gene, termed HER2 (human EGFR-related gene), resulted in the discovery that the gene encoding the HER2/neu receptor tyrosine kinase is amplified up to 100 fold in tumor cells of about 30% of patients with invasive breast cancer.

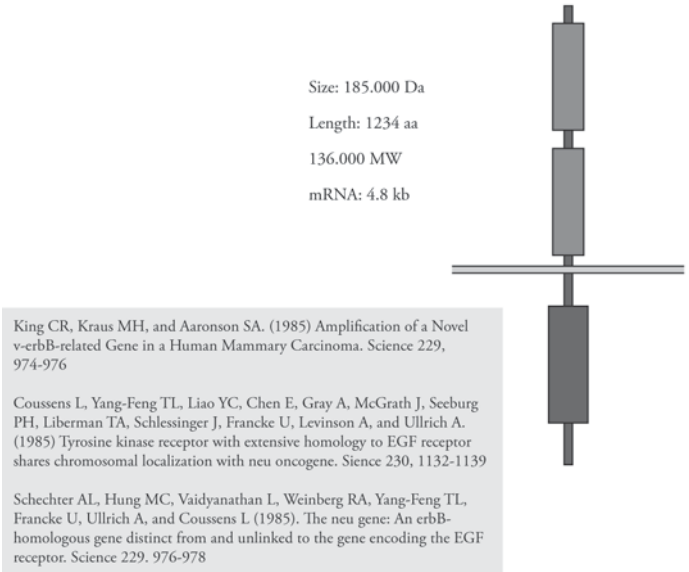

Fig 2: Human EGF Receptor-Related Receptor HER2/neu

A significant clinical correlation was shown between HER2/neu gene amplification and overexpression and parameters of malignancy, including reduced survival and reduced time to relapse, relative to patients with normal receptor levels. Along this line we have elucidated the primary structures of some very

important Growth Factors by cDNA cloning of the precursor proteins for EGF, NGF, IGF-1 and IGF-2[6,7].

At the same time this work initiated a new era of the "war against cancer". The homology of EGFR and v-erb-B, an established oncoprotein from the avian erythroblastosis virus, revealed the potential role of growth factor receptors in human cancers.

So the first step in the "war against cancer" was the cloning and primary structure elucidation of the first signal transducing cell surface protein, the EGF receptor which turned out to be a Growth Factor Receptor Tyrosine Kinase and a Proto-Oncogene. This was the first complete characterization of a human proto-oncogene (c-erb-B) product with known biological function which was followed by the discovery of EGFR gene amplification in cancer cells. The first identification of oncogenic mutations has been recognized to be critical for human cancer[5].

This work was continued by cloning and characterization of receptors for insulin (IR), PDGF, IGF-1, CSF-1 and SCF which are survival factors for cancer cells and by elucidation of oncogenic mutations in CSF-1 and SCF (kit) receptors. With these results we have confirmed the existence of animal oncogene-related human proto-oncogenes by cloning the human homologs of v-fms and v-kit and thereby, at a very early stage, long before the "field" picked up this track, estab-

[6] Ullrich, A., Gray, A., Berman, C., and Dull, T.J. (1983) Human beta-nerve growth factor gene sequence highly homologous to that of mice. *Nature* 303 (5920), 821-825.

[7] Ullrich, A., Berman, C.H., Dull, T.J., Gray, A., and Lee, J.M. (1984) Isolation of the human insulin-like growth factor I gene using a single synthetic DNA probe. *EMBO J.* 3 (2), 361-364.

lished "cancer targets" for therapy development. All but the IR currently serve as targets for cancer drug development[8,9,10,11].

Probably the most important result of this area beside elucidation of the EGFR sequence was the discovery of the EGFR-related gene, HER2/c-erbB2, encoding a putative receptor with an unknown ligand. HER2 turned out to be the human homolog of the oncogene neu which established another "cancer connection" of the receptor tyrosine kinase gene family.

The ultimate goal was to fight human cancer with new weapons. In 1985 I initiated collaboration with *Dennis Slamon,* an oncologist at UCLA, with the intention to investigate the occurrence of animal oncogenes like v-erb-B in human tumors. Genomic analysis of primary human breast tumors with Slamon using our cDNA probes - EGFR, HER2, EGF, PDGF, IGF1, IGF2, NGF - led to the discovery that the gene encoding HER2 is amplified and overexpressed in 25% of all breast cancers and that HER2 amplification predicts rapid disease progression. In my laboratory then we developed several monoclonal antibodies against HER2, two of which, MAb 4D5 and 2C4 were subsequently humanized and developed by Genentech for therapeutic use.

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[8] Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y-C., Tsubokawa, M., Mason, A., Seeburg, P.H., Grunfeld, C., Rosen, O.M., and Ramachandran, J. (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313 (6005), 756-761.

[9] Yarden, Y., Escobedo, J.A., Kuang, W-J., Yang-Feng, T.L., Daniel, T.O., Tremble, P.M., Chen, E.Y., Ando, M.E., Harkins, R.A., Francke, U., Fried, V.A., Ullrich, A., and Williams, L.T. (1986) Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature* 323 (6085), 226-232.

[10] Coussens, L., Van Beveren, C., Smith, D., Chen, E., Mitchell, R.L., Isacke, C.M., Verma, I.M., and Ullrich, A. (1986) Structural alteration of viral homologue of receptor proto-oncogene fms at carboxyl terminus. *Nature* 320 (6059), 277-280.

[11] Yarden, Y., Kuang, W-J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T.J., Schlessinger, J., Francke, U., and Ullrich, A. (1987) Human proto-oncogene c-kit: A new cell surface receptor-tyrosine kinase for an unidentified ligand. *EMBO J.* 6 (11), 3341-3351.

The discovery of the growth factor receptor HER2/neu and the demonstration of its major role in mammary cancer progression has become a basic milestone in Target-Specific Cancer Therapy and brought along the First Personalized Medicine approach. This was achieved by the development of HER/neu-specific monoclonal antibody 4D5, which was subsequently humanized to become the first cancer genomics-based, personalized, target-specific anti-oncogene cancer therapeutic -Herceptin- (Genentech Inc. / Hoffmann-La Roche) and has been available to breast cancer patients since 1998.

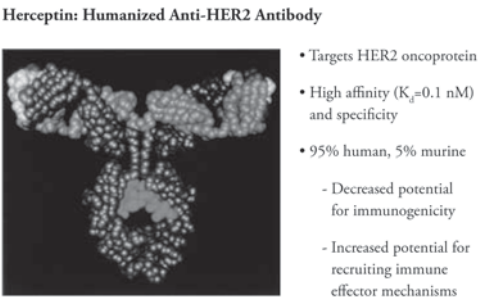


Fig 3: Herceptin: Humanized Anti-HER2 Antibody

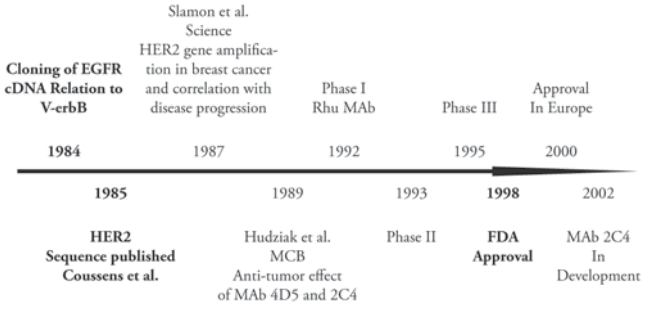


Fig 4: Herceptin history

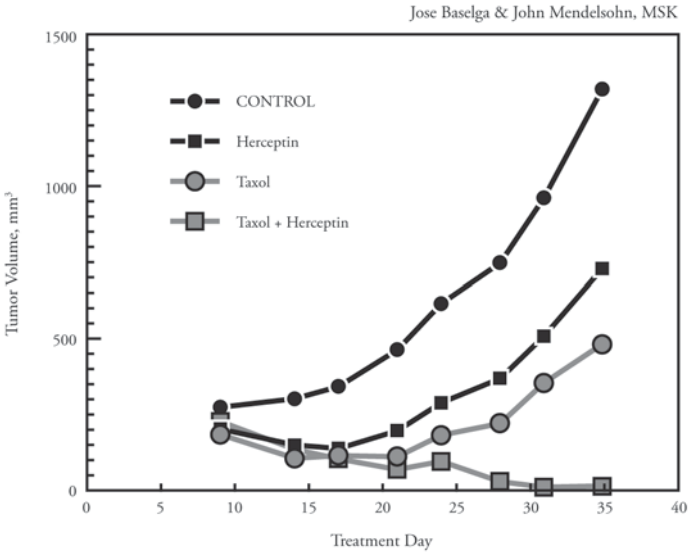


Fig 5: Treatment of BT474 xenografts with Herceptin and Taxol

In 2005 results of a multi-center trial (HERA) which were reported at ASCO demonstrated a major benefit for HER2 positive breast cancer patients in adjuvant therapy preventing tumor recurrence in 46% of the probands[12,13,14,15,16].

[12] Coussens, L., Yang-Feng, T.L., Liao, Y-C., Chen, E., Gray, A., McGrath, J., Seeburg, P.H., Libermann, T.A., Schlessinger, J., Francke, U., Levinson, A., and Ullrich, A. (1985) Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 230 (4730), 1132-1139.

[13] Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A., and McGuire, W.L. (1987) Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235 (4785), 177-182.

[14] Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., and Press, M.F. (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244 (4905), 707-712.

[15] Hudziak, R.M., Lewis, G.D., Winget, M., Fendly, B.M., Shepard, H.M., and Ullrich, A. (1989) $p185^{HER2}$ monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. *Mol. Cell. Biol.* 9 (3), 1165-1172.

[16] Fendly, B.M., Winget, M., Hudziak, R., Lipari, M.T., Napier, M.A., and Ullrich, A. (1990) Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER-2/neu gene product. *Cancer Res.* 50 (5), 1550-1558.

Herceptin (trastuzumab) for the treatment of metastatic breast cancer and Gleevec (imatinib mesylate from Novartis) for the therapy of patients with Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) are the first examples of gene-based cancer drugs, and represent the most significant development toward a new era of target-directed therapies. These agents not only prolong life and improve its quality, they also provide clinical validation of the emerging field of molecular oncology, specifically therapies targeting kinase enzymes that play a critical role in tumorigenesis and –malignant progression.

Following the prototypical example of Herceptin, drugs that interfere with the disease-promoting functions of these receptors are currently being developed by pharmaceutical companies worldwide. In direct continuation of his work that led to Herceptin we developed MAbs against HER3, a co-signal transducer of EGFR and HER2, which is currently in phase II clinical trials by U3 Pharma/ Daiichi-Sankyo[17].

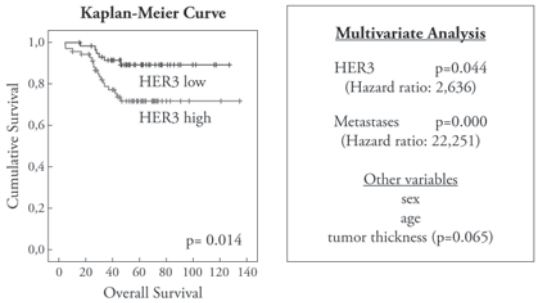


Fig 6: HER3 expression confers poor prognosis for melanoma patients

[17] Htun-van der Horst, E., Murgia, M., and Ullrich, A. (2005) Anti-HER-3 Monoclonal Antibodies Inhibit HER-3-mediated Signaling in Breast Cancer Cell Lines Resistant to Anti-HER-2; Anti-HER-3 Monoclonal Antibodies in Breast Cancer Therapy. *Int. J. Cancer* 115 (4), 519-527.

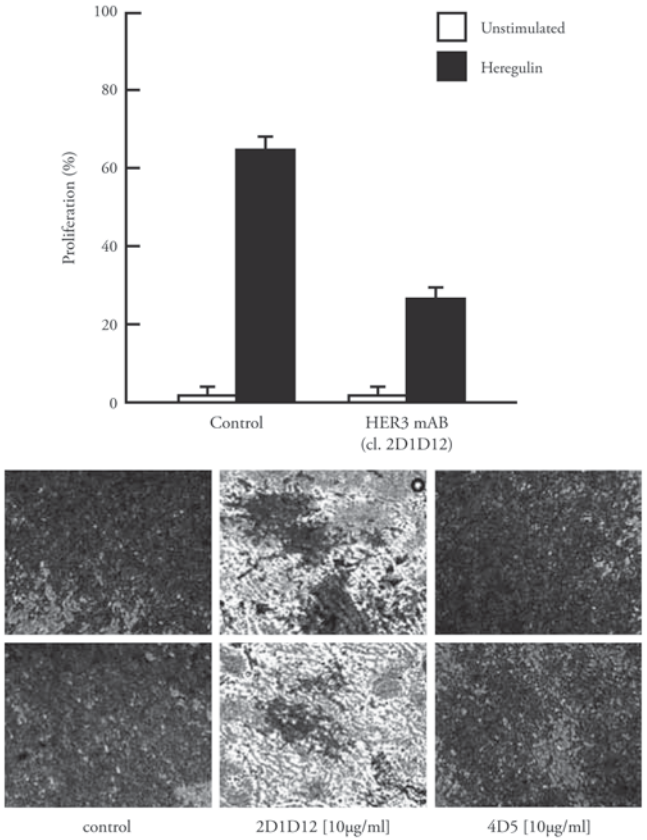


Fig 7: Proliferation and invasion inhibition by HER3maB 2D1D12

In the 20 years since the isolation of the cDNA encoding the epidermal growth factor receptor (EGFR) and the deduction of its amino-acid sequence, intensive research efforts have led to important insights into the molecular mechanisms of receptor tyrosine kinase (RTK) function and involvement in cancer.

Moreover, substantial advances have been made in understanding the key roles of RTKs in the signaling pathways that govern fundamental cellular pro-

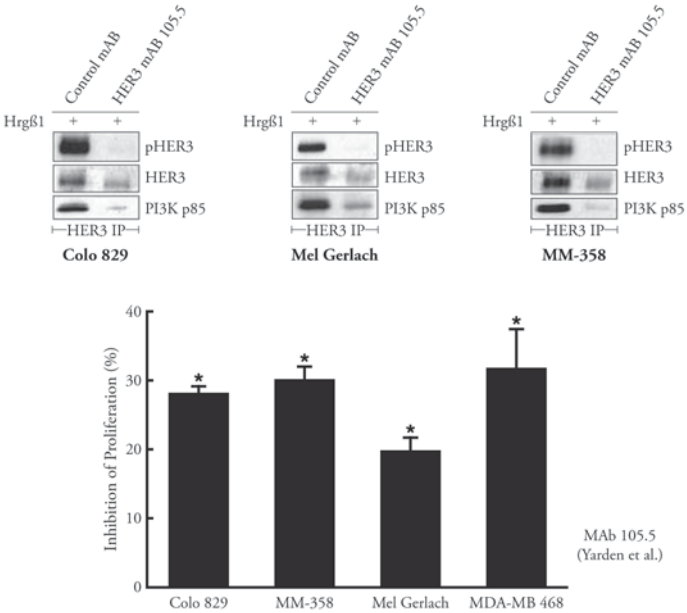


Fig 8: Anti-HER3 antibodies inhibit HER3 activation and melanoma cell proliferation

cesses, such as proliferation, migration, metabolism, differentiation and survival, as well as those that regulate intercellular communication during development. RTK activity in resting, normal cells is tightly controlled. When these genes are mutated or structurally altered, however, RTKs become potent oncoproteins: abnormal activation of RTKs in transformed cells has been shown to be causally involved in the development and progression of many human cancers. Consequently, RTKs and their growth-factor ligands have become rational targets for therapeutic intervention using humanized antibodies and small molecule drugs. In recent years, RTK-based cancer therapies — for example, for the treatment of metastatic breast cancer, kidney cancer, gastrointestinal stromal tumors and non-small cell lung can-

cer — h a v e

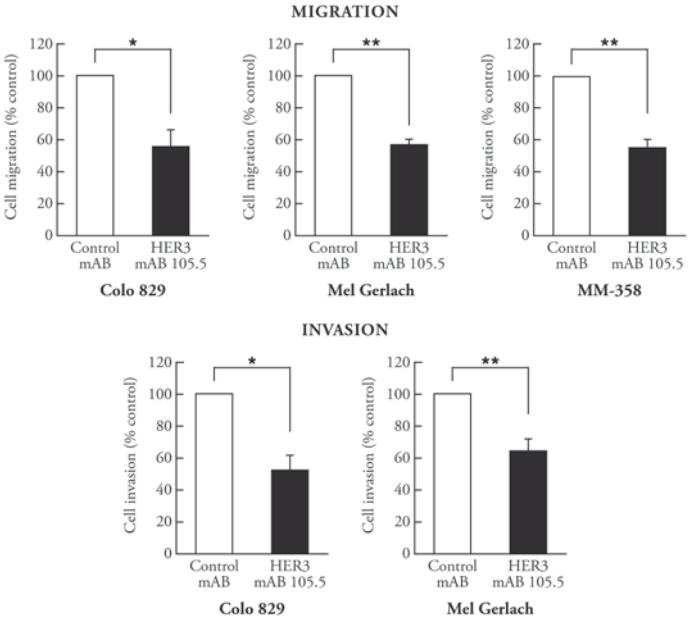


Fig 9: Anti-HER3 antibody 105.5 inhibits melanoma cell migration and invasivity

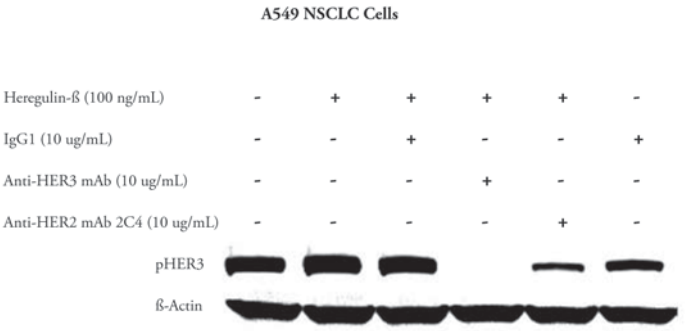


Fig 10: human anti-HER3 mAb inhibits ligand-induced HER3 activation

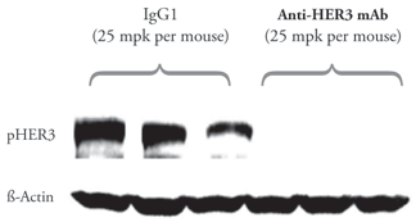


Fig 11: Treatment with human anti-HER3 mAb significantly reduces pHER3 levels in BxPC-3 tumor xenografts

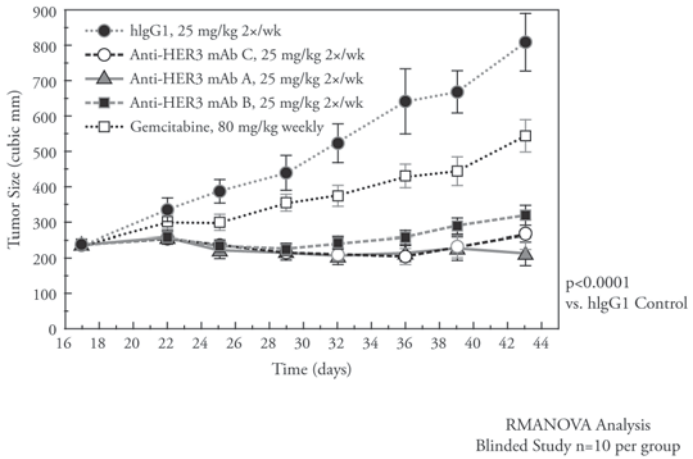


Fig 12: Treatment with human anti-HER3 mAbs results in inhibition of BxPC-3 pancreatic carcinoma xenografts

reached widespread clinical use and have thereby demonstrated the power of gene-based therapy development[18].

The strategy of genomics-based, target-driven drug development has revolutionized medicine and has led to new "bullets in the war against cancer" like

[18] Gschwind, A., Fischer, O. M. and Ullrich, (2004) A. The discovery of receptor tyrosine kinases: targets for cancer therapy *Nature Reviews Cancer* 4: 361-370.

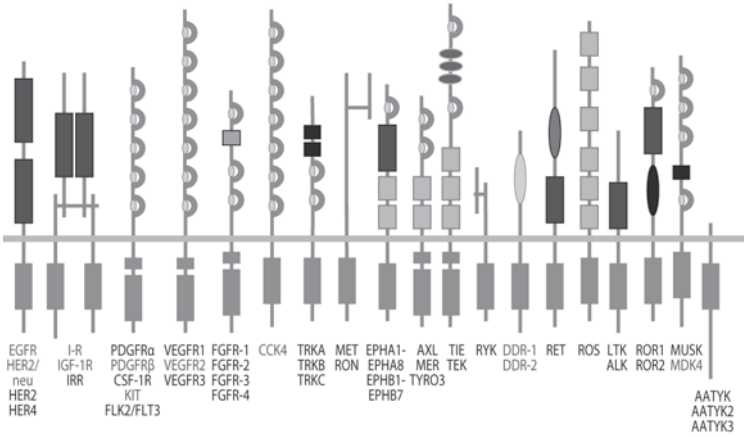


Fig 13: RTK subclasses

Gleevec, Iressa, Tarceva, Nexavar and others. Analogous approaches towards "Personalized Signal Transduction Therapies for Cancer" are now being pursued by hundreds of academic and industrial laboratories worldwide.

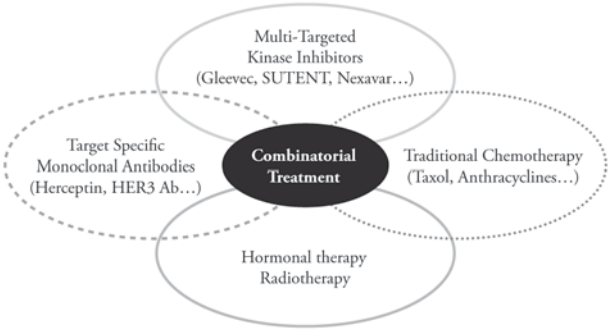


Fig 14: Future of individualized cancer therapy

In the 1990s after moving to the Max Planck Institute of Biochemistry in Martinsried (Germany) I continued to initiate the development of yet more ef-

ficacious cancer therapies by further exploring the significance of the phosphotyrosine signaling system and the kinases controlling it.

We have discovered and functionally characterized the receptor tyrosine kinase Flk-1/VEGFR2 as an essential element of the process of tumor angiogenesis and by this finding have initiated another important strategy for cancer therapy.

We demonstrated that blocking this receptor in a rat brain tumor model resulted in significantly impaired tumor growth. This anti-angiogenic approach was subsequently brought to clinical application by Sugen Inc., a biotech company co-founded by me. Collaborating with György Kéri's company Biosignal utilizing his library we have identified the chemical structures of oxindols which strongly inhibit the Flk-1/VEGFR2 kinase.

The identification of Flk-1/VEGFR2 as the critical receptor for the development of the vascular system and for tumor angiogenesis and the identification of its chemical inhibitors (in close collaboration with Alex Levitzki of Hebrew University and György Kéri of Vichem Ltd.) resulted in the development of the target-specific (Flk-1/VEGFR2) anti-angiogenic drug (SU5416) and the multi-targeted drugs SU6668 and SU11248 by SUGEN, Inc. for the treatment of various cancers.

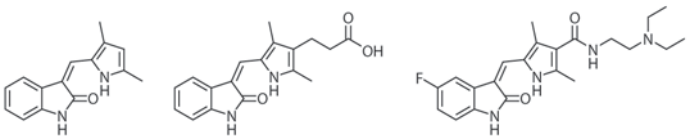
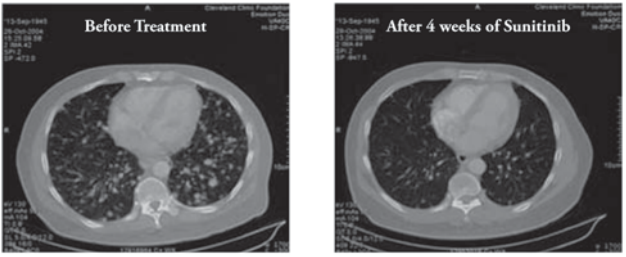


Fig 15: From mono- to multi-targeted kinase inhibitors

SU11248 (SUTENT) was submitted in July 2005 (Pfizer) to the FDA for approval for the treatment of Gleevec-resistant Gastrointestinal Stromal Tumors (GIST).

SU11248/SUTENT/Sunitinib, the first "designed" multi-targeted cancer drug (Pfizer), was approved on January 26, 2006 for the treatment of GIST and Renal Cell Carcinoma by the FDA and in July 2006 by the European EMEA[19,20,21,22,23].



Lung Lesion Response in RCC;
Courtesy of Dr. Ronald Bukowski, Cleveland Clinic Foundation

Sunitinib is New First-Line Therapy in Metastatic Cell Renal Cell Carcinoma (RCC)
Adverse Side Effects are Tolerable

Fig 16: Sunitinib in metastatic RCC

[19] Millauer, B., Shawver, L.K., Plate, K.H., Risau, W., and Ullrich, A. (1994) Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1 mutant. *Nature* 367 (6463), 576-579.

[20] Millauer, B., Longhi, M.P., Plate, K.H., Shawver, L.K., Risau, W., Ullrich, A., and Strawn, L.M. (1996) Dominant-negative inhibition of Flk-1 suppresses the growth of many tumor types in vivo. *Cancer Res.* 56 (7), 1615-1620.

[21] Strawn, L.M., McMahon, G., App, H., Schreck, R., Kuchler, W.R., Longhi, M.P., Hui, T.H., Tang, C., Levitzki, A., Gazit, A., Chen, I., Kéri, G., Orfi,, L., Risau, W., Flamme, I., Ullrich, A., Hirth, K.P. and Shawver, L.K. (1996) Flk-1 as a Target for Tumor Growth Inhibition. *Cancer Res.* 56 (15), 3540-3545.

[22] Fong, T.A.T., Shawver, L.K., Sun, L., Tang, C., App, H., Powell, T.J., Kim, Y.H., Schreck, R., Wang, X.Y., Risau, W., Ullrich, A., Hirth, K.P., and McMahon, G. (1999) SU5416 is a potent and selective Inhibitor of the Vascular Endothelial Growth Factor Receptor (Flk-1/KDR) that Inhibits Tyrosine Kinase Catalysis, Tumor Vascularization, and Growth of Multiple Tumor Types. *Cancer Res.* 59 (1), 99-106.

[23] Laird, A.D., Vajkoczy, P., Shawver, L.K., Thurnher, A., Liang, C., Mohammadi, M., Schlessinger, J., Ullrich, A., Hubbard, S.R., Blake, R.A., Fong, A.T., Strawn, L.M., Sun, L., Tang, C., Hawtin, R., Tang, F., Hirth, K.P., McMahon, G., and Cherrington, J. (2000) SU6668 is a potent Anti-Angiogenic and Anti-Tumor Agent that Induces Regression of Established Tumors. *Cancer Res.* 60 (15), 4152-4160.

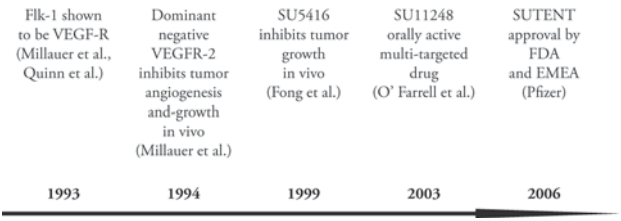


Fig 17: Sutent history

Sunitinib represents a prototype multi-targeted therapeutic that promises to be efficacious in many different cancers as recently shown for neuroendocrine pancreatic tumors.

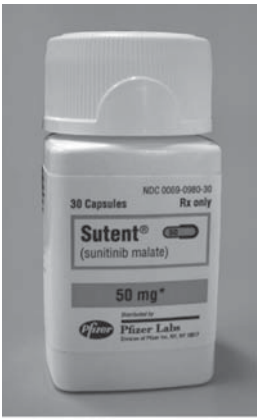


Fig 18: Sutent

Further exploring the significance of the phospho-tyrosine signaling system and the kinases controlling it we have validated the Axl/Ufo Receptor Tyrosine Kinase as a Cancer Drug Development Target and discovered that this tyrosine kinase is a major metastasis-promoting cell surface signal transducer in Glioma,

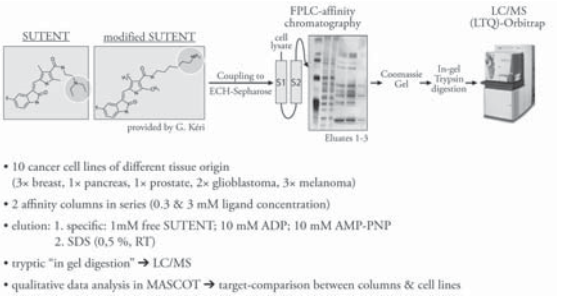


Fig 19: Target-identification via affinity chromatography and mass spectrometry

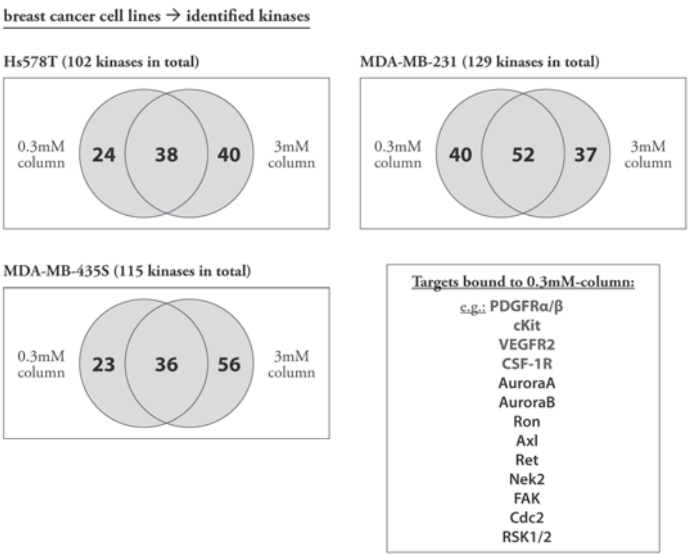


Fig 20: Kinase affinity spectrum in cancer cell lines

breast cancer[24] and other cancers. To identify the genes that mediate progression of breast cancer we have focused on key elements of the phosphor-protein-

[24] Vajkoczy, P., Knyazev, P., Kunkel, A., Capelle, H.-H., Behrndt, S., von Tengg-Kobligk, H., Kiessling, F., Eichelsbacher, U., Knyazev, P., Essig, M., Read, T.-A., Erber, R., and Ullrich, A. (2006)

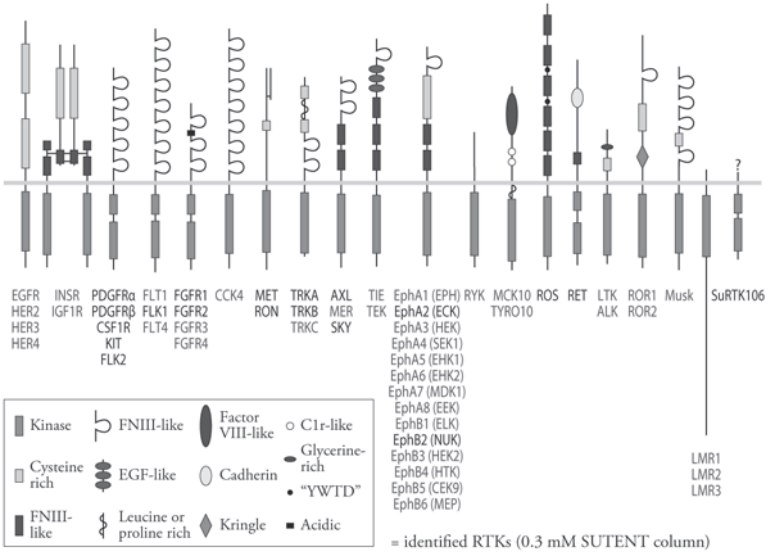


Fig 21: Sutent-responsive RTKs

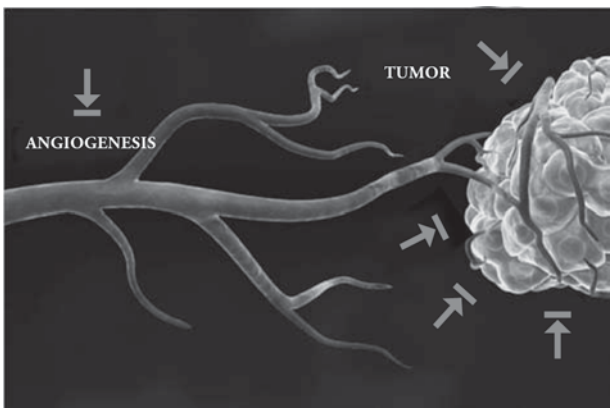
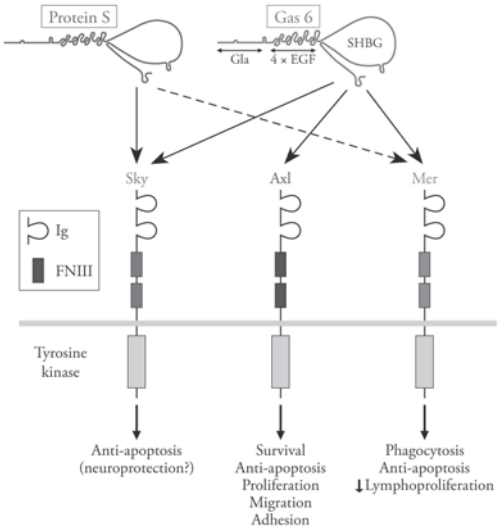


Fig 22: Sunitinib acts on angiogenesis and tumor cells

Dominant-negative Inhibition of the Axl Receptor Tyrosine Kinase Suppresses Brain Tumor Cell Growth and Invasion, and Prolongs Survival. *Proc. Natl. Acad. Sci.* 103 (15), 5799-5804.

mediated signaling system because of its established role in human cancer. After systematically analyzing expression profiles of kinases of 13 weakly invasive and 8 highly invasive breast cancer cell lines and normal mammary epithelia cell lines by cDNA array hybridization analysis, we identified a cluster of genes characteristic for highly invasive cell types. The RTK AXL was part of the gene cluster, predictive of the aggressiveness of breast cancer cells.



Hafizi S, *et al.*. Cytokine Growth Factor Rev. 2006, 17(4): 295-304

Fig 23: Gas/AXL system

The mammalian AXL RTK subfamily includes three closely related members: AXL, SKY, and MER. GAS6, originally isolated as a growth arrest–specific gene, is the common ligand for AXL subfamily receptors. GAS6-AXL signaling has been implicated in a host of discrete cellular responses including cell survival, proliferation, migration, and adhesion.

AXL expression has been reported in a wide variety of human cancers[15,16,17,19,24,25].

Especially in breast cancer patients, a significant correlation was found between AXL and tumor stage[15]. Moreover, some reports indicated that AXL might be involved in cancer progression[20,21]. These prompted us to further investigate the role of AXL in breast cancer. Experimental inhibition of AXL signaling by a dominant-negative AXL mutant, an antibody against the extracellular domain of AXL, or short hairpin RNA knockdown of AXL decreased motility and invasivity of highly invasive breast cancer cells.

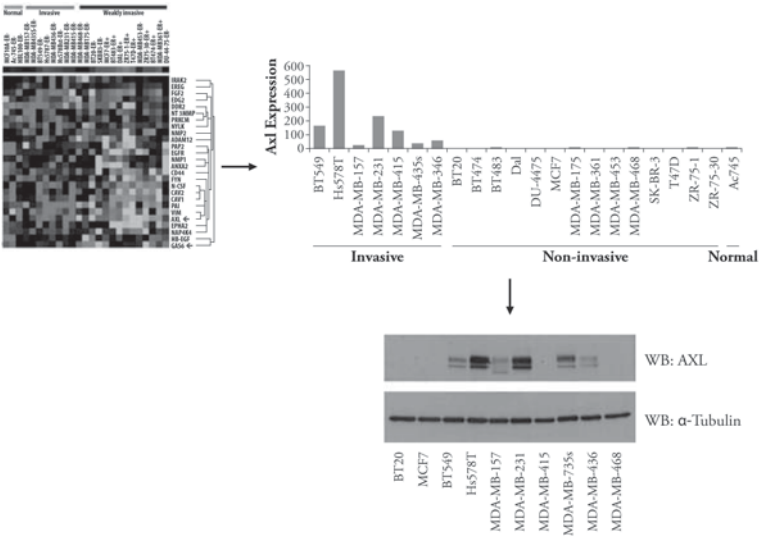


Fig 24: Identification of AXL as a gene characteristic for invasive breast cancer

To selectively interfere with cancer cell properties defining the rate of disease progression we identified 3-quinolinecarbonitrile compounds, which displayed potent inhibitory activity against AXL and showed strong interference with motility and invasivity of breast cancer cells. Our findings validated the RTK AXL as

a critical element in the signaling network that governs motility and invasivity of breast cancer cells, and allowed the identification of experimental anti-AXL small molecular inhibitors in collaboration with G. Kéri, L. Orfi and Vichem Chemie that represent lead substances for the development of antimetastatic breast cancer therapy[25].

We have shown that AXL has an important role in mediating breast cancer cell motility and invasivity. Moreover, we identified 3-quinolinecarbonitrile compounds that displayed potent inhibitory activity against AXL, and showed strong interference with motility and invasivity of breast cancer cells. We have developed a series of very potent and drug like Axl kinase inhibitors which are now in preclinical development.

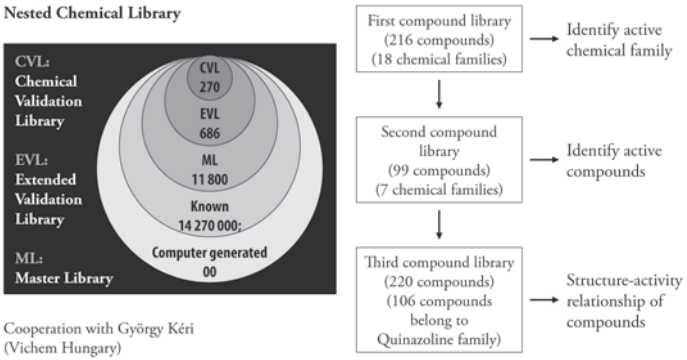


Fig 25: Screening for AXL inhibitors

We have also analyzed the Cellular Signaling Networks in Cancer Cells and discovered and characterized the G-protein-coupled EGF Receptor Transactiva-

[25] Zhang, Y., Knyazev, P. G., Cheburkin, Y. V., Sharma, K., Knyazev, Y. P., Örfi, L., Szabadkai, I., Daub, H., Kéri, G., and Ullrich, A. (2008) AXL Is a Potential Target for Therapeutic Intervention in Breast Cancer Progression. *Cancer Research* 68 (6), 1905-1915.

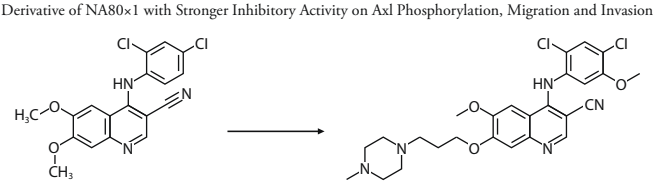


Fig 26: Development of SKI-606

tion pathway in normal and cancer cells and demonstrated that physiological factors like LPA, Angiotensin II, Endothelin and others may be involved in cancer progression[26,27,28,29,30].

In a novel approach with the discovery of a frequent polymorphism in the transmembrane domain of the FGFR4 we embarked on an ambitious cancer genomics endeavour – the investigation of the impact of SNPs on cancer progression, susceptibility, resistance and therapy response. With his discovery of the frequent (50%) FGFR4 388R allele and demonstration of its progression-enhancing effect on breast-, colon, lung and other cancers we have moved towards a new frontier of individualized multi-specific cancer diagnosis and therapy. Via demonstrating the prognostic significance of the FGFR4Arg388 allele for breast-

[26] Daub, H., Weiss, F.U., Wallasch, C. and Ullrich, A. (1996) Role of transactivation of the EGF receptor in signaling by G-protein-coupled receptors. *Nature* 379 (6565), 557-560.

[27] Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R. Wallasch, C. and Ullrich, A (1999) EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402 (6764), 884-888

[28] Schäfer, B., Gschwind, A., and Ullrich, A. (2004) Multiple G-Protein-Coupled Receptor Signals Converge on the Epidermal Growth Factor Receptor to Promote Migration and Invasion. *Oncogene* 23 (4), 991-999.

[29] Fischer, O.M., Hart, S., Gschwind, A., Prenzel, N., and Ullrich, A. (2004) Oxidative and Osmotic Stress Signaling in Tumor Cells is Mediated by ADAM Proteases and HB-EGF. *Mol. Cell Biol.* 24 (12), 5172-5183.

[30] Schäfer, B., Marg, B., Gschwind, A., and Ullrich, A. (2004) Distinct ADAM Metalloproteinases Regulate G Protein Coupled Receptor-Induced Cell Proliferation and Survival. *J. Biol. Chem.* 279 (46), 47929-47938.

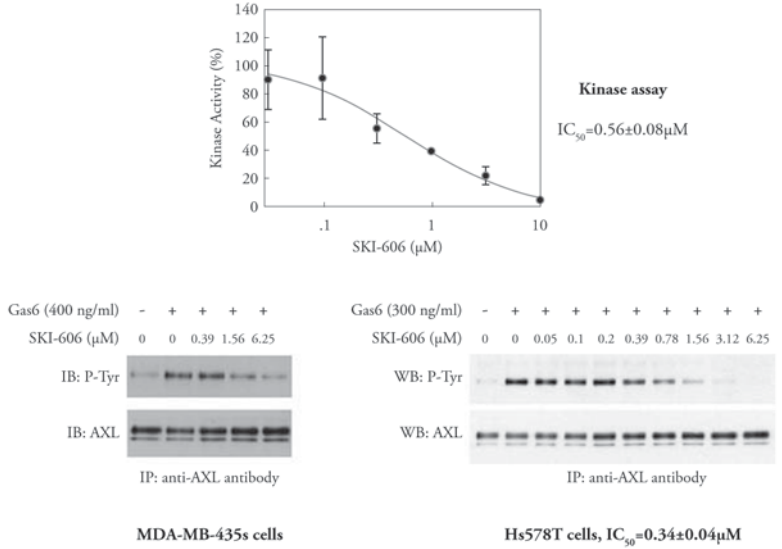


Fig 27: Inhibitory effects of SKI-606 on the phosphorylation of AXL

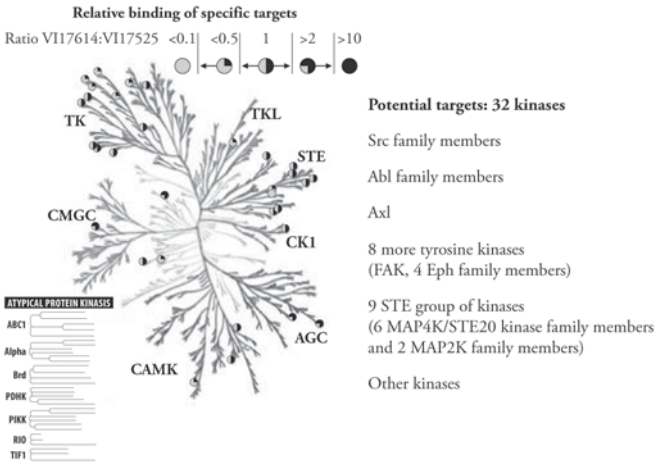


Fig 28: Cellular specificity profile of compounds VI17525 and VI17614

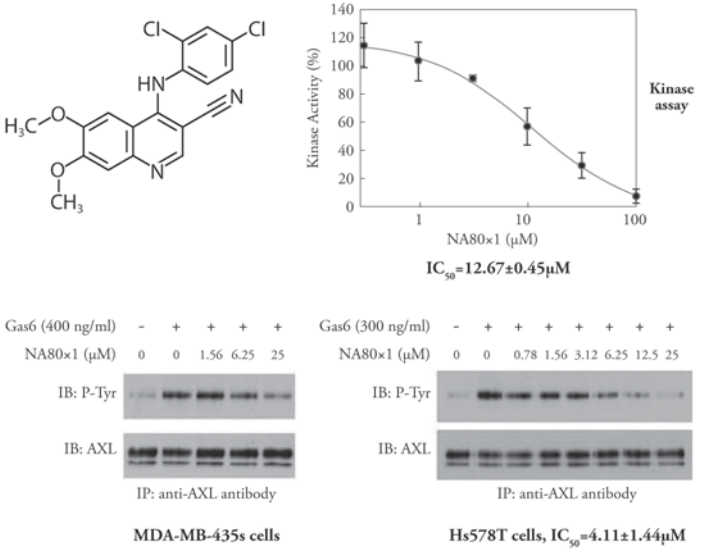


Fig 29: Inhibitory effects of NA80x1 on the phosphorylation of AXL

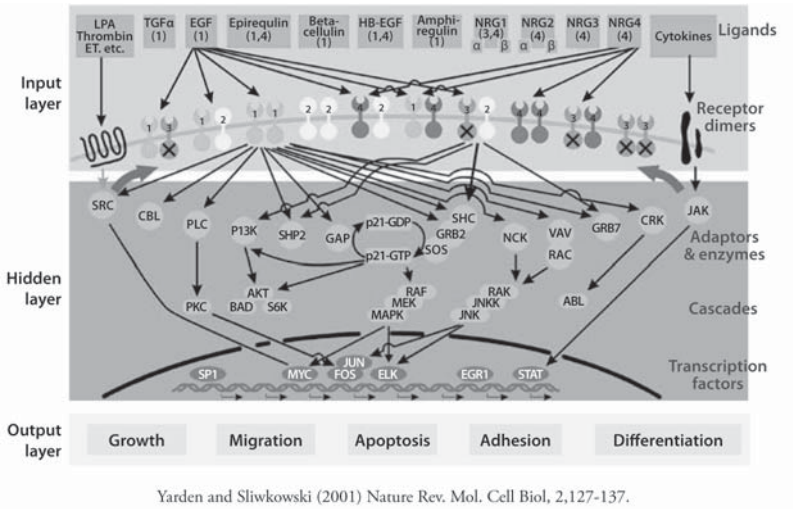


Fig 30: The EGFR family signaling network

and colon cancer patients we have established FGFR4 as an intervention target for cancer therapy[18,31,32,33].

We have shown that FGFR4 gene expression is up-regulated in doxorubicin-treated, apoptosis resistant cancer cell clones. Ectopic expression of FGFR4 in cancer cells led to reduced apoptosis sensitivity on treatment with doxorubicin or cyclophosphamide, whereas knockdown of endogenous FGFR4 expression in breast cancer cell lines had the opposite effect. FGFR4 overexpression resulted in Bcl-xl up-regulation at both mRNA and protein levels. Knockdown of FGFR4 expression by small interfering RNA caused a decrease in phospho-extracellular signal-regulated kinase1/2 levels and reduced Bcl-xl expression.

Fibroblast growth factor receptor-4 (FGFR4) is a tyrosine kinase with a range of important physiological functions. However, it is also frequently mutated in various cancers and is now generating significant interest as a potential therapeutic target. Unfortunately, biochemical characterization of its role in disease and further evaluation as a drug target is hampered by lack of a specific inhibitor. We aimed to discover new inhibitors for FGFR4 using a strategy combining in silico, in vitro and cell-based assays. We used the homologous FGFR1 to calculate docking scores of a chemically-diverse library of approximately 2000 potential kinase inhibitors.

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[31] Bange, J., Prechtel, D., Cheburkin, Y., Specht, K., Harbeck, N., Schmitt, M., Knyazeva, T., Müller, S., Gärtner, S., Sures, I., Wang, H., Imyanitov, E., Häring, H.U., Knyazev, P., Iacobelli, S., Höfler, H. and Ullrich, A. (2002) Cancer progression and tumor cell motility are associated with the FGFR4 Arg388 allele. *Cancer Res.* 62 (3), 840-847.

[32] Thussbas, C., Nährig, J., Streit, S., Bange, J., Seebauer, M., Kates, R., Ulm, K., Kiechle, M., Höfler, H., Ullrich, A., and Harbeck, N. (2006) FGFR4 Arg388 Allele is Associated with Resistance to Adjuvant Therapy in Primary Breast Cancer. *J. Clin. Oncol.* 24 (23), 3747-55.

[33] Streit, S., Mestel, D.S., Schmidt, M., Ullrich, A., and Berking, C. (2006) FGFR4 Arg388 Allele Correlates with Tumor Thickness and FGFR4 Protein Expression with Survival of Melanoma Patients. *Br. J. Cancer* 94 (12), 1979-1886.

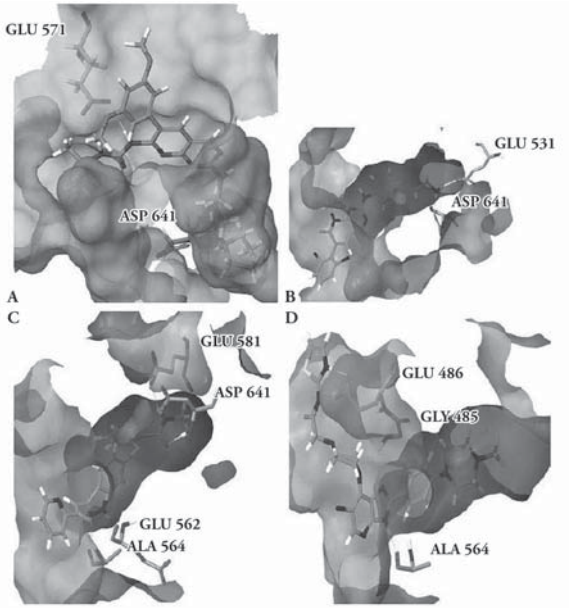

Fig 31: Docking poses of representative structures

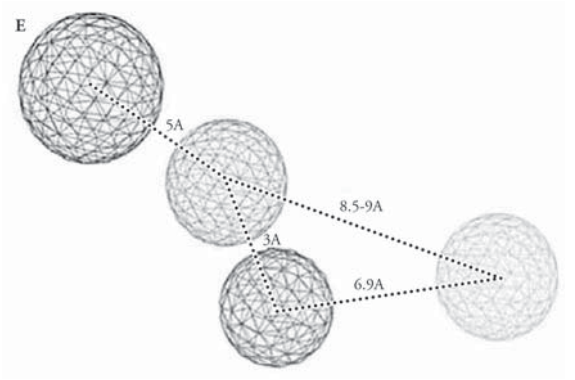

Fig 32: Identified pharmacophore points

Nineteen potential inhibitors and ten randomly selected negative controls were taken forward for in vitro FGFR4 kinase assays. All compounds with good docking scores significantly inhibited FGFR4 kinase activity, some with sub-micromolar potency (most potent being V4-015 with an IC_{50} of 0.04 μM). Four of these compounds also demonstrated substantial activity in cellular assays using the FGFR4-overexpressing breast carcinoma cell line, MDA-MB453.

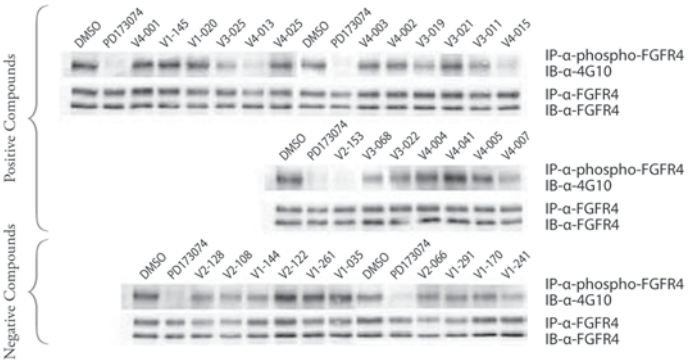

Fig 33: FGFR4 phosphorylation is inhibited by various compounds in MDA-MB453 cells

Through immunoblot assays these compounds were shown to block the phosphorylation of the FGFR4 adaptor protein, FGFR substrate protein-2α (FRS2α).

The most potent compound to date, V4-015, suppressed proliferation of MDA-MB453 cells at sub-micromolar concentrations, activated the pro-apoptotic caspases 3/7 and inhibited cellular migration.

While achieving complete selectivity of this compound for FGFR4 will require further lead optimization, this study has successfully identified new chemical scaffolds with unprecedented FGFR4 inhibition capacities that will support mechanism of action studies and future anti-cancer drug design.

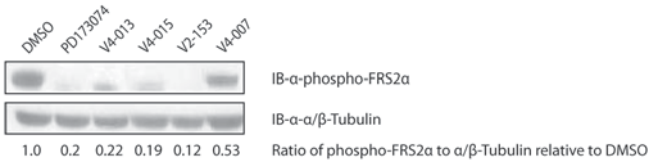

Fig 34: FRS2α phosphorylation is reduced by potential FGFR4 inhibitors in MDA-MB453 cells

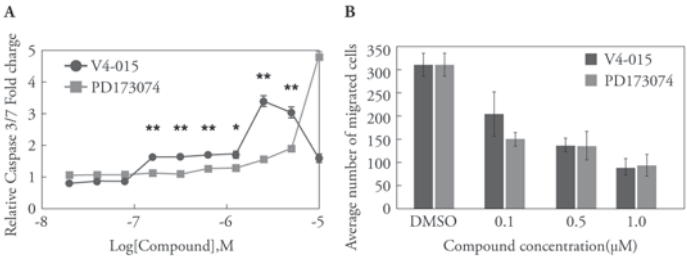

Fig 35: V4-015 induces apoptosis and inhibits cell migration in breast cancer cell lines

Summarising our results in molecular medicine between 1977 and 2013 we have pioneered the translation of genomics-based discoveries into novel approaches for the treatment of major diseases such as Cancer and Diabetes. With two cancer therapeutics and one for the treatment of diabetes that have emerged from this scientific work and several promising translational programs under development we have made significant contributions to the field of translational medical research worldwide.