

NEWER TARGETS FOR CANCER: A REVIEW

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Summary

Cancer is the leading cause of death worldwide. Over 2 million people in the UK alone are currently living with the consequences of cancer and its treatment and this figure is projected to grow at more than 3% in a year. However, despite major improvements in diagnostic tools, patient management and cytotoxic therapies during the past quarter century, the impact on long-term survival has been modest. As our knowledge of the molecular biology of cancers in general has increased exponentially during the last 2 decades, multiple new targets have been identified and they provide a host of new approaches. This review addressed some of the recent approaches and idea for the treatment of cancer.

Keywords: Cancer, tumorigenesis, malignancies, therapeutic targets.

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Introduction

With over 2 to 2.5 million cases at any point of time in India, Cancer has become one of the leading causes of death in the country. Over 7 lakh new cases & 3 lakh deaths occur every year due to cancer in India. This figure is much bigger worldwide. By 2020, there are expected to be 15 million new cases of cancer every year globally, 70% of which will be in developing countries. In view of grim situation at hand, it is imperative that research in this field be pursued with renewed vigor. The past decade has seen host of molecular & genetic targets being identified in this field, though the number of molecules entering clinical trials has reduced generally [1].

As described in their path breaking article by Hanahan et al., 2000, virtually all kinds of cancers have been found to possess 6 hallmarks namely (1) Self-sufficiency in growth signals (2) Insensitivity to anti-growth signals (3) Evasion of apoptosis (4) Limitless replicative potential (5) Sustained angiogenesis (6) Tissue invasion and metastasis. Thus, during the course of its transformation from a normal cell to a tumor cell, the cell acquires all of the above traits, ultimately bypassing the defense mechanisms of host cell. It has now been established that a number of changes at genetic level are responsible for these changes [2].

While conventional therapeutic approaches to cancer have been limited in their efficacy and safety. Thus, more emphasis is being laid on targeted therapy. As indicated further in this discussion, recent discoveries have increasingly indicated genomic changes to be vital linkage in all varieties of cancer. Tumorigenesis as a process has been connected to genetic alterations which ultimately transform normal cells to malignant. Most of recently identified targets for cancer therapy are “oncogenes” i.e. genes whose mutation or over-expression may potentially lead to different forms of cancers. A “Proto-oncogene” on the other hand, is a normal gene which has the potential to turn into oncogenes. The future direction of cancer therapy thus, will be focused on trying to inhibit the conversion of a Proto-oncogene to an Oncogene, or to limit its over-expression once it is converted [3].

Data Search & Review Criteria:

For the purpose of this review article, we focused on relevant publications available online, as well as few offline sources. A conscious effort was made to include studies published after 1995. We tried being comprehensive & did not restrict data by any specific criterion. Some of the web based sources consulted were PUBMED, Google Scholar, the lancet etc. The search terms included were newer, trends, cancer, malignancies, targets, oncogenes etc.

Emerging Targets in Anti Cancer Therapy

Aurora Kinases:

Aurora Kinases are a family of enzymes (Serine/Threonine Kinases), over expression of which have been linked to various types of malignancies. They are so named because of the scattered mitotic spindles generated by their mutant forms resemble the *Aurora Borealis*. These are supposed to play a vital role in cellular mitosis, there various roles including spindle formation, chromosome alignment etc. The family has been classified into 3 subtypes viz. Aurora – A, B & C, among which the role of Aurora-C in the development of cancer has not yet been confirmed. Among these, Aurora-A, also name AURKA has been most extensively studied. Aurora-A maps to human chromosome 20q13.2, while Aurora Kinase-B or AURKB has been mapped to chromosome 17q13 [4, 5].

Due to their significant role in division of cancerous cells & significant over-expression in various malignancies, interest has been developing recently for the development of Aurora Kinase inhibitors. Inhibition of Aurora A & B in tumor cells has been established to impair chromosomal alignment, which subsequently leads to cell death [6].

Some of the molecules undergoing clinical trials in U.S. & Europe are-VX-680 (Vertex Pharmaceuticals), PF-03814735 (Pfizer), AZD-1152 (Astra Zeneca) & PHA-739358 (Nerviano Medical Sciences). VX-680, later renamed as MK 0457 (Merck) or Tozasertib (structure shown in figure 1), a molecule under joint development by Vertex Pharmaceuticals and Merck, was the first molecule from this category to enter clinical trials & is in advanced stages (Phase II clinical trial). BI811283, AZD1152 & GSK1070916 are inhibitors specific for AURORA-B Kinase [7]. MLN-8054 & MLN-8254 (Millenium), ATP-competitive Aurora Kinase Inhibitors have been discovered recently. Preliminary clinical data of these molecules have shown promising results in an otherwise treatment refractory population. A significant reasonable therapeutic index has

been indicated, though toxicity related to cell division in rapidly dividing tissues of the body, such as hematopoietic system is not uncommon. Among different phase-1 studies, Neutropenia has emerged as primary toxicity, suggesting the effect of these molecules on bone marrow cell proliferation [4, 8].

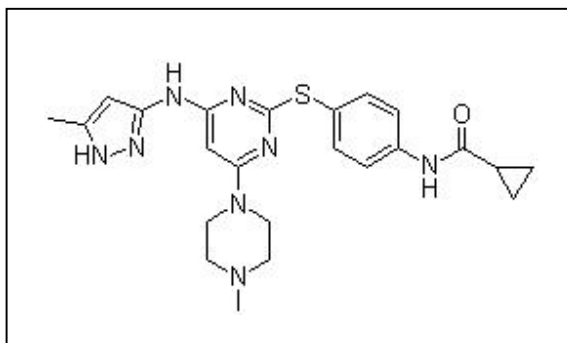


Figure 1. Structure of Tozasertib (MK-0457) [9]

Aurora Kinase Inhibitors have shown a marked specificity for acting on the cells entering mitosis, a feature which makes them distinctly advantageous over traditional anti-mitotic agents like Vinka alkaloids & Taxanes. However, their effects on solid tumours as concluded by recent clinical findings have been disease stabilizing at best [9]. Future strategies are directed towards using them in combination with cytotoxic agents or radiotherapy.

Role of Insulin like growth factor (IGF) Pathways:

The IGF system consists of 3 ligands (Insulin, IGF-1 & IGF-2), which bind to 4 receptors. IGF-1R, a tyrosine kinase receptor, is being perceived as a crucial target for potential anti-cancer therapies. IGF-1 is produced by the Liver in response to release of Growth Hormone (GH) by the Pituitary gland, during Puberty. This IGF-1 plays an important role in childhood growth.

IGF-1 signaling via the IGF-1R receptor has been confirmed in various steps of the cell cycle viz. cell proliferation, differentiation, motility etc. Besides IGF-1R receptor has been associated uniquely with anchorage independent growth in cells, a property which implicates their role in tumorigenesis. This has been confirmed further by studies showing increase in risk factor for breast cancer during higher plasma levels of IGF-1 [10].

Various approaches are thus being tried including receptor blockade & reducing expression as well as synthesis of the receptor. The first monoclonal antibodies targeting IGF-1R are already under clinical trials. Another strategy involves developing Growth Hormone antagonists, to reduce the production of IGF-1. A promising compound of this category- Pegvisomant, which was usually administered in acromegaly, showed significant anti-tumour properties. Other than these, molecular inhibitors as well as disruptors of IGF-1R have been developed.

Along with their role in Glucose metabolism, IGF-1R is involved in a host of normal body functions including maintenance of cardiac function, bone formation & hematopoiesis to name a few. Thus, inhibition of IGF-1R may lead to toxicities too, due to disruption of above processes, most important being Hyper-insulinemia or Hyper-glycemia, which is definitely undesirable for a long period of time. Thus, an allowable risk-benefit ratio or a greater therapeutic window is being targeted through the ongoing clinical trials [11, 12].

Role of mammalian Target of Rapamycin (mTOR):

The mTOR genes are a group of protein kinase enzyme coding genes, which have been named on the basis of the molecule inhibiting their function. Rapamycin is a macrocyclic lactone obtained from *Streptomyces hygroscopicus* & inhibits mTOR intracellularly. TOR was first identified in yeast *Saccharomyces cerevisiae*, following which their mammalian analogue (mTOR) was identified. mTOR has been established to be implicated in a variety of malignancies viz. osteosarcoma, B-cell lymphoma, neuroblastoma etc. It is mainly involved with protein synthesis along with controlling cell growth & proliferation [13]. Primary structure of mTOR is shown in figure 2.

It has been observed that most of the upstream & downstream signaling molecules for mTOR were activated during cancers, establishing its role. In addition; Rapamycin induced inhibition of mTOR led to arrest of cell cycle in G-1 phase, indicating role of mTOR in tumorous cell division. It has also been suggested that, since Insulin is major upstream effector of mTOR, any deficiency in mTOR signaling may play a role in development of Type-2 Diabetes. Thus, effects of mTOR inhibition are being observed curiously by the scientific community [13, 14].

Analogues of Rapamycin under clinical trials include RAD001 Everolimus/RAD 001 (Novartis), Temsirolimus/CCI-779 (Wyeth-Ayerst), and Deforolimus/AP23573 (Ariad Pharmaceuticals) [15]. Everolimus & Temsirolimus have been approved by USFDA for various cancers, while Deforolimus entered phase-III clinical trials in 2008 [16, 17].

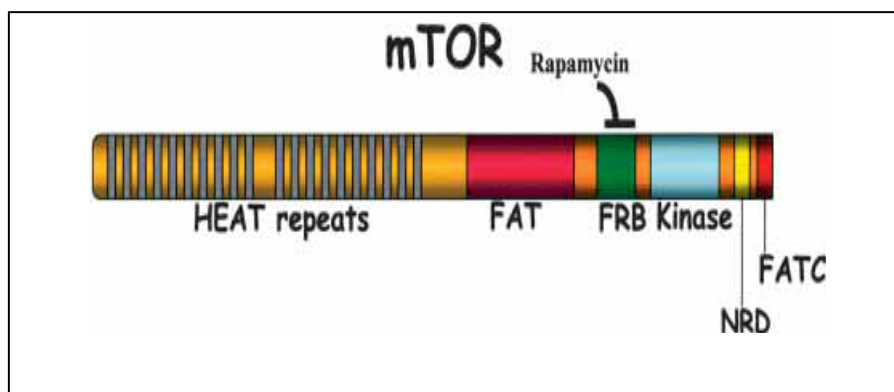


Figure 2. Primary structure of mTOR [13].

Micro RNAs (mi RNA):

First discovered in 1990's, mi RNA, present a fascinating new field in cancer research. These are generally 18-24 nucleotide long RNAs, indicated to play important roles in cellular differentiation & proliferation. These small RNA molecules help regulate gene expression by repressing the translation of target genes by suppressing the function of different messenger RNAs. Their role in development of cancers was first established in a study focusing on B-cell chronic lymphocytic leukemia, where deletion of two miRNAs –miR-15a & miR-16-1 was linked to development of this disease. miR-21, another micro RNA was found to be significantly over-expressed in Glioblastoma cell lines & its inhibition led to increased apoptosis in these cell lines. Other studies linking miRNAs to breast, lung & colorectal cancer have also surfaced [18].

Further studies have justly classified miRNAs as being either Proproliferative/Anti-Apoptotic, which are found to be over expressed in certain cancers, or Anti-Proliferative/Proapoptotic, based on their being under expressed in certain malignancies. Thus, a miRNA may be tumorigenic or tumour suppressive based on above classification. Although some miRNAs are produced at abnormally high levels in cancer cells, the levels of most miRNAs are reduced in tumors, an example being liver cancer, in which significantly lower levels of miR-26a were observed. This led to a significant finding at NIH, USA where therapeutically increasing levels of miR-26a resulted in reduced cancer cell proliferation. Understanding the exact roles of various miRNA in different forms of cancers, hence offers large incentives in terms of understanding the pathogenesis of such cancers & possibly finding cures for the same [19, 20].

IRF (Interferon Regulatory Factors): New target for Multiple Myeloma

IRFs are a group of transcription factors involved in immunological processes like activation of lymphocytes & generation of plasma cells. They are expressed in lymphoid cells, dendritic cells, and macrophages, where they are associated with regulation of important cellular processes, including cell differentiation, apoptosis, DNA repair, and cytokine production. It is believed that they exert their action by activating various genes involved in the above cellular processes. So far, nine members of the IRF family have been discovered & accordingly named IRF 1-9. Among these, oncogenic potential has been best observed in IRF-2 & IRF-4 [21].

Multiple myeloma, a cancer of the blood system, originates in antibody-producing cells called plasma cells, which develop from immune cells called B cells. In a recent study, the researchers showed that turning off IRF4 gene causes myeloma cells to die. It was reported that the IRF4 protein controls the activity of a large network of genes in myeloma cells playing an important role in cell growth and proliferation and in the development of myeloma and other cancers. In addition, over expression of IRF-4 is linked to the pathogenesis of adult T-cell leukemia and lymphoma, though other additional factors seem to be necessary for oncogenic activity. These findings thus, reveal an otherwise unknown target for this critical disease [22, 23]. Other studies linked changes in expression of IRF-1 & IRF-2 to development of Breast cancer. Further studies are underway, finding the possible role of these factors in other malignancies [24, 25].

The Breast cancer associated gene 1 (BRCA 1 gene) & role of Poly ADP-ribosyl polymerase (PARP):

Women who inherit a defective version of the BRCA1 or have greatly increased risks of breast, ovarian, and other cancers. Up to 60 percent of women with a defective BRCA1 gene will develop breast cancer during their lifetime, and 15 to 40 percent will develop ovarian cancer. Thus far, no effective therapies have been developed that overcome the susceptibility to cancer caused by mutations in BRCA1. However, an emerging class of molecules termed “PARP inhibitors” has shown tremendous promise against BRCA 1/2 related breast cancers [26].

The characteristic flaw in BRCA-1 deficient tumor cells is their inability to repair DNA damages like DNA double strand breaks, making them susceptible to the action of DNA damaging anti-cancer drugs like Alkylating agents. However, the action of such drugs is not limited to cancerous cells, resulting in their toxicity. This is where the class of PARP inhibitors comes in.

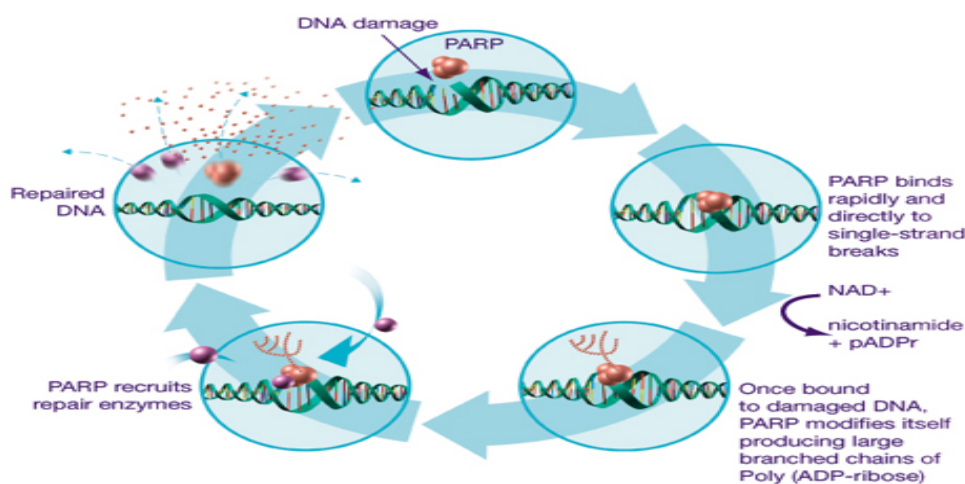


Figure 3. Role of PARP in DNA damage repair [27].

“PARP” is a protein involved in DNA repair process. Characterized into two subtypes- PARP 1 & PARP 2, this family of enzymes activates during DNA damage caused during biological processes such as repair & recombination, as well as during damage caused due to oxidative stresses or DNA binding drugs & are now considered an essential factor in DNA recovery process. To express the function of PARP in a nutshell, PARP are involved in NAD^+ dependent formation of polymers of ADP-ribosyl, which then play an important role in spatial organization of DNA repair, by loosening the chromatin meshwork allowing access to DNA for subsequent repair involving other enzymes [28].

Many of existing therapies against cancer focus on DNA strand damage in cancerous cells, for ex. Alkylating agents or Radiation, while PARP on the other hand are involved in the repair of DNA damage caused by these agents. Inhibition of PARP can thus, be used as a strategy to sensitize the tumor cells for the action of such agents. This was confirmed by using PARP-1 knockout mice which were found to be hypersensitive to the action of alkylating agents & radiations.

Inhibition of PARP-1 ultimately results in formation of DNA double strand breaks (DSB). In BRCA 1/2 deficient cancers, cancerous cells cannot repair such DNA DSBs properly, which arrests their growth & subsequently leads to cell deaths. Normal cells however are able to do such repair, which accounts for higher specificity against such tumors by PARP-1 inhibition [26, 28, 29]. Role of PARP and BRCA in DNA damage repair is shown in figure 3 & 4 respectively.

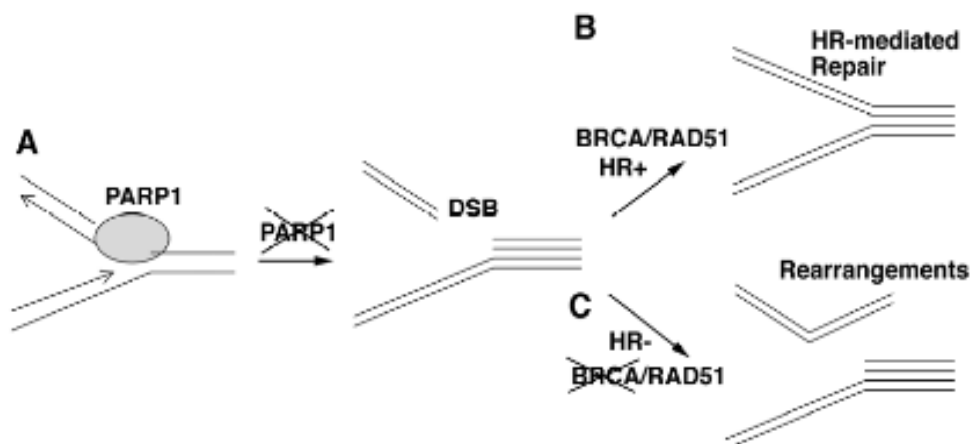


Figure 4. Role of PARP & BRCA in DNA damage repair [28]. (A) PARP repairs DNA Single strand breaks. In case of PARP inhibition, these single strand breaks convert to double strand breaks (DSB). (B) In presence of BRCA, these DSBs get repaired via RAD51, while in BRCA deficient tumor cells, this repair is hampered.

Neurokinin-1 receptor:

A member of G-Protein coupled family of receptors, NK-1 receptor have recently emerged as vital linkages in development of variety of tumors. After binding to Substance-P, a neuropeptide, it has been established to take part in tumor growth, metastasis & angiogenesis. In addition, inhibitors of NK-1 have been shown to inhibit tumor cell growth & proliferation. The SP/NK-1 system is involved in a variety of normal body functions viz. arterial vasodilation, gastric mobility, regulation of cardiac function etc. Studies have shown the tumor cells to have higher number of NK-1 receptors than normal cells, so much so that the numbers of NK-1 receptors present are being used as a criterion in determining the spread of malignancy [30].

Distributed over a variety of chemical classes, a plethora of agonists & antagonists for the NK-1 receptor have been developed. Aprepitant (approved by FDA in 2003), the first NK-1 antagonist developed by MERCK, is being used for chemotherapy-induced emesis. The Second drug in this category, Casopitant has completed phase II & III clinical trials & is indicated for chemotherapy induced, as well as post-operative induced nausea & vomiting. Vestipitant developed by GlaxoSmithKline, is another selective antagonist for the NK1 receptor. NK-1 antagonists have been shown to be effective over a wide range of tumorous cell lines including melanoma, neuroblastoma, gastric & colorectal carcinoma cell lines. They have been shown to inhibit cancerous growth by inducing apoptosis. They have also been shown to block metastasis of cancerous tissue as well as inhibit angiogenesis in such tissues. It has been postulated that inhibition of NK-1 receptors leads to blockage of signals by SP inducing mitosis, leading to eventual cell death [31].

A combination of NK-1 antagonists with other available anticancer agents such as Cisplatin, Cyclophosphamide as well as Radiation was reported to be synergistic & found to reduce the side effects associated with chemotherapy & radiation. Another important feature is the relatively better safety profile of these agents- the prototype Aprepitant was found to be relatively well tolerated at daily dose of 300 mg. In light of the above features regarding safety & relative specificity of these agents, NK-1 receptor antagonists hold great potential to become successful anti-cancer remedy in future [32].

The Epidermal Growth Factor Receptor (EGFR) Family:

The EGFR, a transmembrane tyrosine kinase receptor, plays an important role in cellular proliferation & DNA synthesis. By means of similar mode of action, over-expression of this receptor in cancerous cells may leads to proliferation of cancerous cells, angiogenesis, blockage of apoptosis & so on. Discovered back in 1980s, this receptor has been implicated in most of epithelial cancers. The EGFR interacts with three other tyrosine kinase receptors- HER2, HER3 & HER4 to perform its functions [33]. Effector pathway of EGFR tyrosine kinase receptor is shown in figure 5.

EGFR antagonists have now been under development for more than 20 years, the first compound being Gefitinib. Two approaches have been tried for EGFR antagonism, the first being development of monoclonal antibodies (Cetuximab), which cause competitive inhibition of the other receptor & the other approach being development of small molecules, which inhibit downstream signaling of the tyrosine kinase receptor intracellularly, like Gefitinib. As many as four EGFR antagonists have been currently approved by the FDA- Erlotinib, Gefitinib, Cetuximab & Panitumumab, & more than 10 anti-EGFR agents are in the pipeline. Cetuximab, in particular, received much attention & has been approved for treatment in metastatic colorectal cancer [34]. So far these agents are being used in Non-small-cell lung cancer, colorectal cancer, pancreatic cancer & squamous cell carcinoma of head & neck.

However, recent clinical trials have suggested appearance of resistance against EGFR inhibitors, possibly by tumor cells acquiring alternative pathways for growth or by somehow activating downstream effectors of these receptors. Both Primary & Acquired resistance have been observed, presenting complications in front of physicians. Nonetheless, EGFR inhibitors present an interesting class of agents for future studies [35-37].

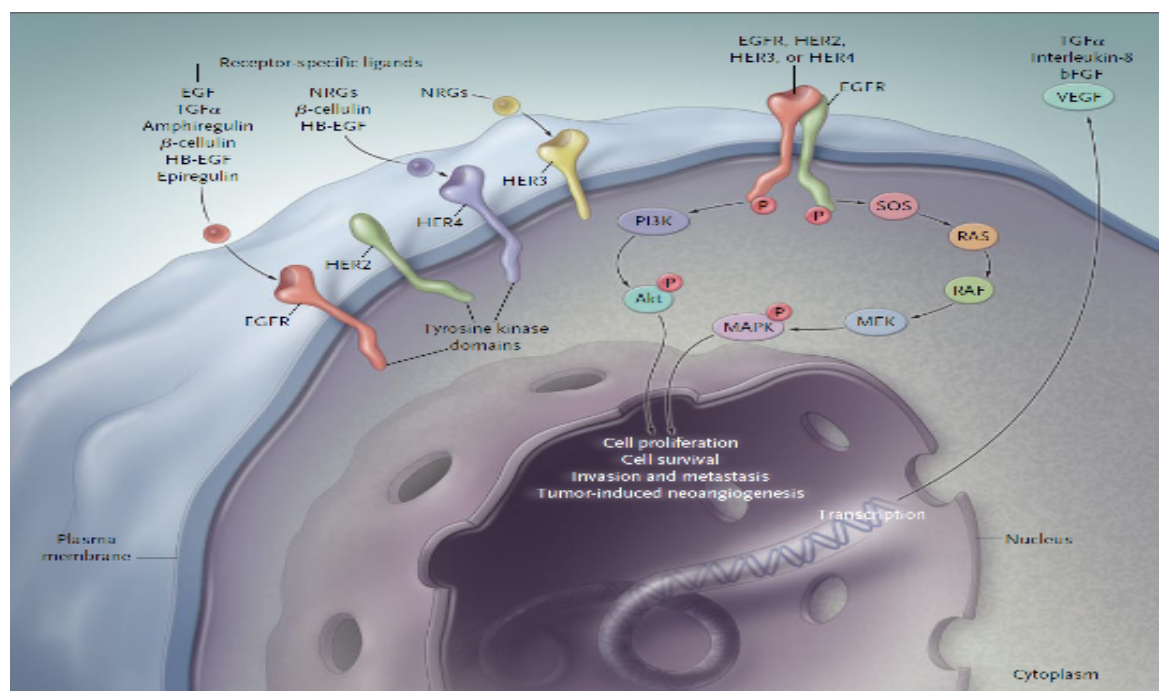


Figure 5. EGFR & related tyrosine receptors, & their effector pathways [35].

Hepatocyte Growth Factor (HGF) & receptor c-met:

Similar to EGFR, the Hepatocyte Growth Factor has been known play important role in cell mitosis & apoptosis. The receptor for HGF is C-Met, a tyrosine kinase receptor. The HGF, identified back in the 1980s has been found to be ubiquitously present in variety of tissues like Kidney, Liver, Lung, Placenta etc. & plays an important role in their repair & regeneration. In vascular endothelial cells, HGF was found to aid in angiogenesis. Similar to other growth factors, HGF has been found to be implicated in aiding tumor growth & metastasis too. Hence, blockage of HGF/c-Met activity creates a new target [38, 39]. HGF Signaling pathways shown in figure 6.

The search for HGF inhibitors led to NK-4, which is only a fragment of HGF molecule & acts as a competitive inhibitor for binding to c-Met tyrosine kinase. The polypeptide HGF is a heterodimeric molecule made up of two chains- α & β . The fragment NK-4, obtained by enzymatic cleavage, is devoid of β subunit & few other amino acids [40]. It acts by inhibiting phosphorylation of the c-Met tyrosine & is specific for c-Met receptor. Experiments with cancerous cell lines showed NK-4 to be useful in a variety of malignancies including prostate cancer, lung cancer, colon cancer etc [41]. Interestingly, NK-4 was shown to inhibit angiogenesis in cancerous tissues. The use of transgenes (U1snRNA/Ribozymes) to inhibit HGF has also been reported [42]. A recombinant protein named Cgen-241A has also been reported as a potent antagonist [43]. There seems to be significant level of interdependency between various growth factors, as indicated by a study where EGFR inhibitors were used to show mitigation of the actions of HGF. Since effective EGFR inhibitors have been already developed, this appears to be an interesting development. Since they are prominently over expressed in variety of tumors, high levels of HGF/c-Met are being perceived as bio markers for tumorous growth [44].

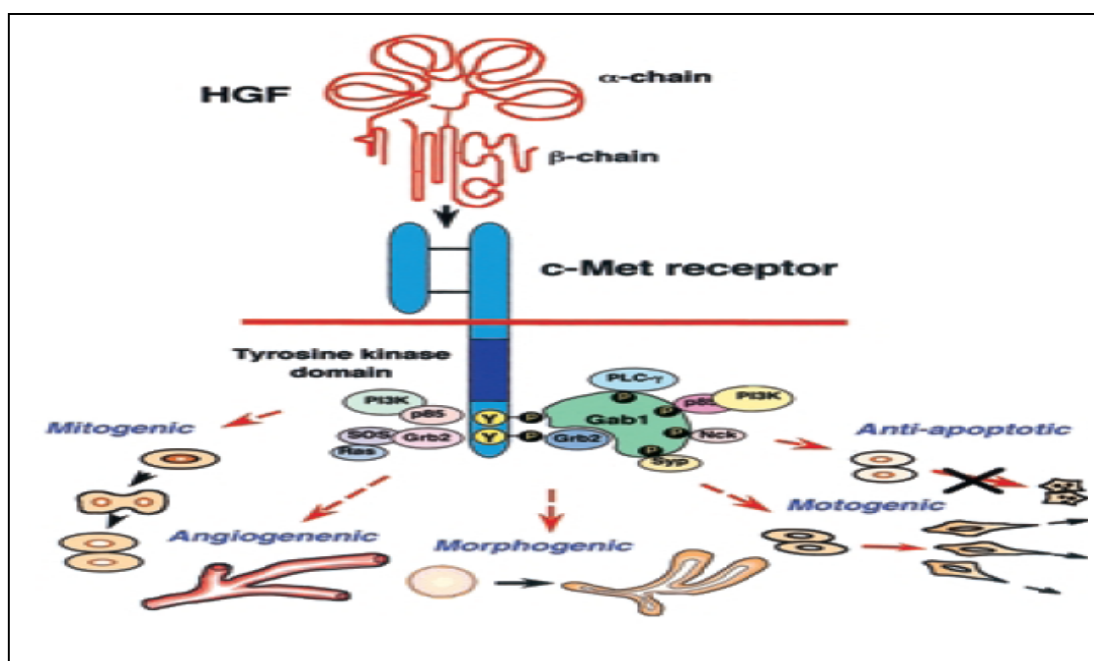


Figure 6. HGF signaling pathways & its major roles in body [38].

Vascular Endothelial Growth Factor (VEGF):

VEGF is another growth factor, which has been known to stimulate angiogenesis & endothelial cell growth, when stimulated by various hormones, cytokines or other factors, such as Hypoxia. It acts via two tyrosine kinase receptors i.e. VEGFR-1 & VEGFR-2. Its role as a potential anti-cancer target was perceived after it was observed that tumor cells had higher levels of VEGF mRNA. It was experimentally proved that cancerous cells produced higher amounts of VEGF as well as over-expressed VEGF Receptor. This over-expression of VEGF is vital for angiogenesis in cancerous tissue, which subsequently fulfills nutritional requirements of this tissue. It has been seen that though VEGF binds to both VEGFR-1 & 2, activation of VEGFR-2 alone is sufficient for angiogenesis [45, 46].

Various strategies are thus being tried for inhibition of VEGF action. These include neutralizing the endogenous ligand, competitive inhibition of receptor & inhibition of tyrosine kinase receptor signaling.

The latter approach is achieved through the development of small molecules capable of obstructing ATP binding to the tyrosine Kinases, thus inhibiting autophosphorylation of the receptors, which is necessary of signal transmission.

This kind of inhibition of VEGF achieves dual purpose of – death of cancerous tissue due to inhibition of growth, & preventing cancer metastases, as it prevents blood vessel development, which is a primary channel for the spread of cancerous tissue [47]. The VEGF family consists of a total of six members, among which VEGF-A, which acts principally via VEGFR-2, has been found to be most involved in angiogenesis in cancerous tissue. Bevacizumab (2004) & Ranibizumab (2006), two monoclonal antibody targeting VEGF have been approved by USFDA [48]. In a separate study, Lovastatin- the HMG-CoA inhibitor was shown to inhibit VEGFR activation, indicating the probable role of mavalonate matabolites in VEGFR activation [49]. VEGFR signaling pathways are shown in figure 7

Conclusion

As indicated in this discussion, the future of research against cancer lies within the realm of genetics. As our understanding of roles of various genes as well as the effector pathways increases, newer targets as well as strategies for treatment of this stubborn ailment will appear. It has been proven so far that combination of existing therapies with newer approaches has yielded best results. Development of small molecules bypassing host defense mechanisms as well as achieving better specificity has been interesting. Though there is much to be learned still, recent developments have raised hopes towards achieving ultimate cure for cancer.

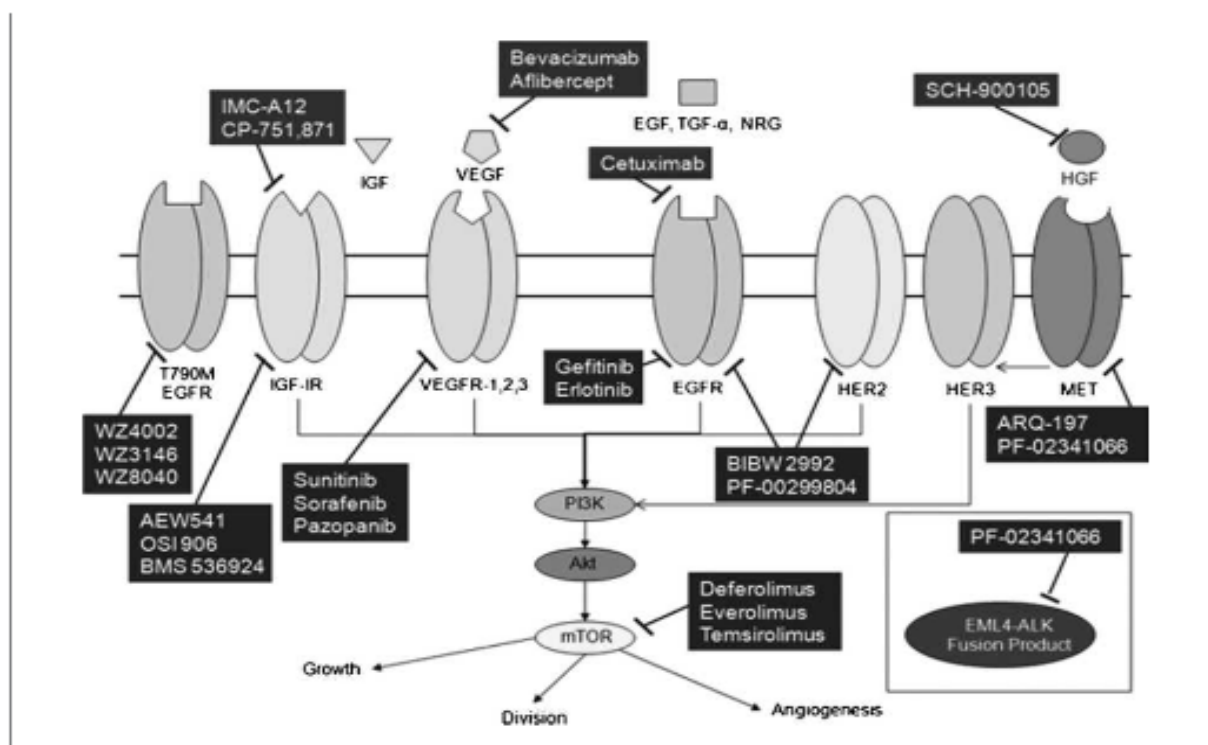


Figure 7. Potential new targets & molecules targeting these targets [50].

References:

1. Parkin D, Pisani P, Ferlay J. Global Cancer Statistics. *CA Cancer J Clin.* 1999; 49: 33-64
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000; 100: 57-70
3. Croce C. Oncogenes and Cancer. *N Engl J Med.* 2008; 358: 502-11.
4. Carvajal RD, Tse A, Schwartz GK. Aurora Kinases: New Targets for Cancer Therapy. *Clin. Cancer Res.* 2006; 12: 6869-6875.
5. Dar A, Goff W. Aurora Kinases' Inhibitors – Rising Stars in Cancer Therapeutics? *Mol Cancer Ther.* 2010; 9(2): 268.
6. Ditchfield C, Johnson V, Tighe A, Ellston R, Haworth C, Johnson T, Mortlock A, Keen N, Taylor S. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J Cell Biol.* 2003; 161: 267-80.
7. Green MR, Woolery JE, Mahadevan D. Update on Aurora Kinase Targeted Therapeutics in Oncology. *Recent Pat Anticancer Drug Discov.* 2008; 3(3): 162-177.
8. Marumoto T, Zhang D, Saya H. Aurora-A: a guardian of poles. *Nat Rev Cancer.* 2005; 5: 42-50.

9. Boss D, Beijnen J. Clinical Experience with Aurora Kinase Inhibitors: A Review. *Oncologist*. 2009; 14(8): 780-93.
10. Hankinson SE, Willett W, Colditz GA, Hunter D, Michaud D, Deroo B, Rosner B, Speizer F, Pollak M. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet*. 1998; 351: 1393–1396
11. Larsson O, Girnita A, Girnita L. Role of insulin-like growth factor 1 receptor signalling in cancer. *British Journal of Cancer*. 2005; 92, 2097–2101.
12. Yee D. Targeting insulin-like growth factor pathways. *British Journal of Cancer*. 2006; 94, 465–468.
13. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004; 18: 1926–1945.
14. O'Reilly KE, Rojo F, She Q, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin D, Ludwig DL, Baselga J, Rosen N. mTOR Inhibition Induces Upstream Receptor Tyrosine Kinase Signaling and Activates Akt. *Cancer Res* 2006; 66:1500-1508.
15. Petroulakis E, Mamane Y, Bacquer OL, Shahbazian D, Sonenberg N. mTOR signaling: implications for cancer and anticancer therapy. *British Journal of Cancer*. 2006; 94, 195–199.
16. Bufalo D, Ciuffreda L, Trisciuglio D et al. Antiangiogenic Potential of the Mammalian Target of Rapamycin Inhibitor Temsirolimus. *Cancer Res*. 2006; 66:5549-5554.
17. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F. Deforolimus (AP23573) a novel mTOR inhibitor in clinical development. *Exp Op on Invest Drugs*. 2008; 17 (12): 1947-1954.
18. Calin G, Ferracin M, Cimmino A, Leva GD et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N. Engl. J. Med*. 2005; 353: 1793–1801
19. Hwang H-W, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *British Journal of Cancer*. 2006; 94, 776–780.
20. Kota J, Chivukula R, O'Donnell K, Wentzel E, Montgomery C, Hwang H, Chang T, Vivekanandan P, Torbenson M, Clark KR, Mendell RJ, Mendell JT. Therapeutic delivery of miR-26a inhibits cancer cell proliferation and induces tumor-specific apoptosis. *Cell*. 2009; 12; 137(6): 1005–1017.
21. Romeo G, Fiorucci G, Chiantore MV, Percario ZA, Vannucchi S, Affabrisirf E. IRF-1as a Negative Regulator of Cell Proliferation. *Journal of Interferon and Cytokine Research*. 2002; 22: 39–47.
22. Shaffer A, Tolga Emre N, Lamy L, Ngo V, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H, Zeng Y, Chen B, Epstein J, Staudt LM. IRF4 Addiction in Multiple Myeloma. *Nature*. 2008; 454(7201): 226–231.
23. Acquaviva J, Chen X, Ren R. IRF-4 functions as a tumor suppressor in early B-cell development. *Blood journal*. 2008; 112: 3798-3806.
24. Bouker BK, Skaar TC, Riggins RB, Harburger DS, Fernandez DR, Zwart A, Wang A, Clarke R. Interferon regulatory factor-1 (IRF-1) exhibits tumor suppressor activities in breast cancer associated with caspase activation and induction of apoptosis. *Carcinogenesis*. 2005; 26(9): 1527–1535.

25. Doherty GM, Boucher L, Sorenson K, Lowney J. Interferon Regulatory Factor Expression in Human Breast Cancer. *Annals of Surgery*. 2001; 233(5): 623–629
26. Me'gnin-Chanet F, Bollet M, Hall J. Targeting poly(ADP-ribose) polymerase activity for cancer therapy. *Cell. Mol. Life Sci*. 2010; 67:3649–3662.
27. Gien LT, Mackay HJ. The Emerging Role of PARP Inhibitors in the Treatment of Epithelial Ovarian Cancer. *Journal of Oncology*. 2010, 1-6.
28. De Soto J, Deng C. PARP-1 inhibitors: are they the long-sought genetically specific drugs for BRCA1/2-associated breast cancers? *Int. J. Med. Sci*. 2006; 3:117-123.
29. Bai P, Canto' C, Oudart H, Brunya' nszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper R, Schoonjans K, Schreiber V, Sauve A, Menissier-de Murcia J, Auwerx J. PARP-1 Inhibition Increases Mitochondrial Metabolism through SIRT1 Activation. *Cell Metabolism*. 2011; 13: 461–468.
30. Esteban F, Muñoz M, González-Moles M, Rosso M. Role for substance P in cancer promotion and progression: a mechanism to counteract intracellular death signals following oncogene activation or DNA damage. *Cancer and metastasis reviews*. 2006; 25 (1): 137-145.
31. Ruhlmann C, Herrstedt J. Casopitant: a novel NK1-receptor antagonist in the prevention of chemotherapy-induced nausea and vomiting. *Therapeutics and Clinical Risk Management*. 2009; 5: 375–384.
32. Muñoz M. Neurokinin-1 Receptor: A New Promising Target in the Treatment of Cancer. *Discov Med*. 2010; 10(53):305-313.
33. Zhang Z, Stiegler A, Boggon T, Kobayashi S, Halmos B. EGFR-mutated lung cancer: a paradigm of molecular oncology. *Oncotarget*. 2010; 25; 1(7): 497–514.
34. Xu L, Hausmann M, Dietmaier W, Kellermeier S, Pesch T, Stieber-Gunckel M, Lippert E, Klebl F, Rogler G. Expression of growth factor receptors and targeting of EGFR in cholangiocarcinoma cell lines. *BMC Cancer*. 2010, 10:302-312
35. Ciardiello F, Tortora G. EGFR Antagonists in Cancer Treatment. *N Engl J Med*. 2008; 358:1160-74.
36. Hasson P, Paroush Z. Crosstalk between the EGFR and other signalling pathways at the level of the global transcriptional corepressor Groucho/TLE. *British Journal of Cancer*. 2006; 94: 771 – 775.
37. Lo H, Hung M. Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. *British Journal of Cancer*. 2006; 94: 184 – 188.
38. Matsumoto K, Nakamura T. NK4 (HGF-antagonist/angiogenesis inhibitor) in cancer biology and therapeutics. *Cancer Sci*. 2003; 94 (4): 321–327.
39. Raymond W, Cruz AC, Caughey GH. Mast Cell and Neutrophil Peptidases Attack an Inactivation Segment in Hepatocyte Growth Factor to Generate NK4-like Antagonists. *J Biol Chem*. 2006; 281(3): 1489–1494.
40. Jiang WG, Hiscox SE, Parr C, et al. Antagonistic Effect of NK4, a Novel Hepatocyte Growth Factor Variant, on in Vitro Angiogenesis of Human Vascular Endothelial Cells. *Clin Cancer Res*. 1999; 5:3695-3703.
41. Du W, Hattori Y, Yamada T, Matsumoto K, Nakamura T, Sagawa M, Otsuki T, Niikura T, Nukiwa T & Ikeda Y. NK4, an antagonist of hepatocyte growth factor

- (HGF), inhibits growth of multiple myeloma cells: molecular targeting of angiogenic growth factor. *Blood*. 2007; 109 (7): 3042-3049.
42. Abounader R, Laterra J. Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. *Neuro-Oncology*. 2005; 7: 436–51.
 43. Tiran Z, Oren A, Hermesh C et al. A Novel Recombinant Soluble Splice Variant of Met Is a Potent Antagonist of the Hepatocyte Growth Factor/Scatter Factor-Met Pathway. *Clin Cancer Res*. 2008; 14:4612-4621.
 44. Bonine-Summers AR, Aakre ME, Brown KA, Arteaga CL, Pietenpol JA, Moses H, Cheng N. Epidermal Growth Factor Receptor Plays a Significant Role in Hepatocyte Growth Factor Mediated Biological Responses in Mammary Epithelial Cells. *Cancer Biology & Therapy*. 2007; 6(4), e1-e10.
 45. Dias S, Choy M, Alitalo K, Rafii S. Vascular endothelial growth factor (VEGF)–C signaling through FLT-4 (VEGFR-3) mediates leukemic cell proliferation, survival, and resistance to chemotherapy. *Blood*. 2002; 99(6): 2179-84.
 46. Valtola R, Salven P, Heikkila P, Taipale J, Joensuu H, Rehn M, Pihlajaniemi T, Weich H, Waal R, Alitalo K. VEGFR-3 and Its Ligand VEGF-C Are Associated with Angiogenesis in Breast Cancer. *Am J Pathol*. 1999; 154:1381–1390.
 47. Ryan A, Wedge S. ZD6474 – a novel inhibitor of VEGFR and EGFR tyrosine kinase activity. *British Journal of Cancer*. 2005; 92(1): S6 – S13.
 48. Midgley R, Kerr D. Bevacizumab—current status and future directions. *Annals of Oncology*. 2005; 16: 999–1004.
 49. Zhao T, Trinh D, Addison C, Dimitroulakos J. Lovastatin Inhibits VEGFR and AKT Activation: Synergistic Cytotoxicity in Combination with VEGFR Inhibitors. *Plos one*. 2010; 5 (9): e12563
 50. Pal SK, Figlin RA, Reckamp K. Targeted Therapies for Non-Small Cell Lung Cancer: An Evolving Landscape. *Mol Cancer Ther*. 2010; 9:1931-1944.