Immunotherapy and colon cancer

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Abstract

Colon cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in both men and women in the U.S. The exact cause of the colon cancer is still unknown but has several risk factors like the age, the colonic polyps, the genetic alterations and others. The aim of this review is to put a light on the pathophysiology of the colon cancer like the genomic and chromosomal instabilities and oncogens mutations like the EGFR; and focus on the immunotherapies available for treating it including the FDA approved agents like Cetuximab, Bevacizumab, Regorafenib, and other drugs that are still under clinical trial.

Abbreviations: HNPCC: Hereditary Non Polyposis Colon Cancer, FAP: Familial Adenomatous Polyposis, APC: Antigen Presenting Cells, CIN: Chromosomal Instability, MMR: Mismatch Repair, CRC: Colorectal Cancer, mCRC: metastatic Colorectal Cancer, CIMP: CpG Island Methylator Phenotype, TGFβ: Transforming Growth Factor B, EGFR: Epidermal Growth Factor Receptor, MAPK: Mitogen Associated Protein Kinase, PI3K: Phosphatidylinositol 3-Kinase, VEGF: Vascular Endothelial Growth Factor, MAb: Monoclonal Antibody, CTL: Cytotoxic T-Lymphocyte, PD-1: Programmed Death 1, mTOR: Mammalian Target of Rapamycin, CIK: Cytokine Induced Killer

Introduction

Colon cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in both men and women in the US [1]. The American Cancer Society estimated 96,830 new cases of colon cancer in the United States in the year 2014. Additionally, in the same year 50,310 cases of death due to colon cancer have been reported [2]. The incidence of colorectal cancer is equal for both men and women. The American Cancer Society estimated that colon cancer was diagnosed in 48,450 men and 48,380 women in the United States in the year 2014 [3].

Although, the incidence of colon cancer varies widely from country to country throughout the world, colon cancer is a common disease in the United States. Recent data on colon cancer in the United States showed that incidence and death due to this cancer is disproportionately higher in African Americans than in whites. Hispanics have the lowest incidence and mortality from colorectal cancer.

The exact cause of colon cancer is still not known. Several risk factors have been found that increases the risk of developing colon cancer. More than 90% of people with colon cancer are diagnosed after the age of 50 and the average age of diagnosis is 72. Generally, polyps grow on the inner wall of colon or rectum. They are more common in people over age 50. Risk of colon cancer increases due to the changes in certain genes [4].

Hereditary nonpolyposis colon cancer (HNPCC) is a type of inherited colon cancer and accounts for about 2% of all colon cancer. Most people with an altered HNPCC gene develop colon cancer, however, it is not just limited to colon cancer, and other organs such as female reproductive organs but also other parts of the gastrointestinal tract, might be affected by malignant transformation. Familial adenomatous polyposis (FAP) is caused by a change in a specific gene called APC. FAP accounts for less than 1% of all colon cancer [4].

A person having colon cancer may develop colon cancer a second time. Women with a history of ovary, uterus or breast cancer are at a higher risk of developing colon cancer. Patients with long term (10 years or more) history of inflammatory bowel disease, either in the form of ulcerative colitis or Crohn’s disease, are susceptible to colon cancer. Diets high in red meat and fat and low in calcium, folate, and fiber increases the risk of colon cancer. Some studies also suggest that a diet low in fruits and vegetables increases the risk of colon cancer. A person who smokes cigarettes may be at increased risk of developing polyps and colon cancer [4].

Pathophysiology and molecular basis

Colon cancer is a disease caused by disrupted growth control. There is an early genetic alteration which takes place in the colonic epithelial cells lining of the bowel wall.

Genomic instability

Loss of genomic stability increases the acquisition of multiple
mutations which leads to the development of colon cancer [5]. It includes chromosomal instability (CIN), microsatellite instability, aberrant DNA methylation and DNA repair defects [5,6].

**Chromosomal instability**

It is found in up to 85% of CRCs [6]. Loss of function of tumour suppressor genes, including APC, whose normal function is to oppose tumorigenesis, has been implicated in the development of chromosomal instability [7]. Loss of function of the APC gene is further associated with familial adenomatous polyposis (FAP), in which hundreds to thousands of adenomatous colon polyps develop, leading to almost 100% lifetime risk of developing colon cancer [8,9].

**Microsatellite instability**

It involves inactivation of genes responsible for DNA mismatch repair (MMR) through somatic mutation or aberrant methylation [6]. This loss of MMR gene function, leads to inability of repairing strand slippage within nucleotide repeats and changes the size of microsatellites [5].

Germine mutations of MMR genes are responsible for Lynch syndrome, or hereditary non-polyposis colorectal cancer (HNPPC), which carries a lifetime risk of CRC of about 80% [5,9]. Mutations leading to loss of function have been identified in four genes involved in MMR: MLH1, MSH2 (accounting for the majority of cases), MSH6 and PMS2. Lynch syndrome is the most common hereditary CRC syndrome accounting for 2%-3% of all cases [8,10].

**Aberrant DNA methylation**

Aberrant methylation of DNA in colon cancer causes loss in the MMR functioning [5,11]. In the CRC genome, there is aberrant methylation within promoter associated CpG islands, leading to silencing of gene expression [5,12]. Hypermethylation of promoters containing CpG islands is known as the CpG island methylator phenotype (CIMP) [6]. This phenomenon is observed in about 15% of CRCs, most of which shows loss of MLH1 expression resulting in MMR deficiency and microsatellite instability [5,13].

**DNA base excision repair genes**

The MYH gene is a base excision repair gene, responsible for repairing DNA damaged by reactive oxygen species [8,14]. Polyposis develops in the presence of germline mutation of both MYH alleles [5]. The mechanism of disease following germline inactivation of MYH is via subsequent somatic mutation of the APC gene, causing chromosomal instability [15].

**Tumor suppressor genes**

TP53 is a key tumor suppressor gene that is mutated in about half of all colorectal cancers [6]. Inactivation of the TP53 gene often coincides with malignant transformation of adenomas [5,6].

Transforming growth factor β (TGFβ) signaling is an important tumor suppressor pathway. Deregulation of this pathway is a frequent observation in colorectal cancers, mediated by inactivating mutations of receptor genes (TGFBR1, TGFBR2) or post receptor signaling pathway genes (SMAD2, SMAD4) [6]. A number of germline mutations, ultimately leading to downregulation of TGFβ signaling, have been reported, including inactivating mutations of SMAD4 [8].

**Oncogenes**

The pathways which exhibit oncogenic mutations in colorectal cancers, include the epidermal growth factor receptor (EGFR), mitogen-associated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3 K) pathway [5,6,16].

EGFR activation triggers an intracellular phosphorylation cascade through downstream effectors RAS and BRAF, amplified through the MAPK pathway to promote cell growth [6]. RAS and BRAF are implicated as oncogenes in a number of human cancers. Activating mutations promoting CRC have been identified in both genes [16]. Mutations in KRAS are found in about 40% of CRCs, occurring as a relatively early event in the adenoma-carcinoma sequence [6,17].

Mutations of the PIK3CA gene, leading to the upregulation of PI3 K signaling, are present in approximately 15%-20% of CRCs. Resulting enhanced prostaglandin E2 synthesis inhibits apoptosis of CRC cells [18].

**Immunotherapy**

Current immunotherapy for colon cancer fall into following categories: monoclonal antibodies, kinase inhibitors, vaccine therapy and adoptive cell therapy.

A. **Monoclonal antibodies**

Colon cancer is one of the GI cancers with existing FDA-approved immunotherapeutic monoclonal antibodies. These include: Cetuximab, which directly inhibits the epidermal growth factor receptor (EGFR), Bevacizumab, which inhibits angiogenesis by directly targeting the vascular endothelial growth factor (VEGF) protein and Panitumumab, which is another EGFR inhibitor.

1. **Cetuximab**: It is a recombinant, human/mouse chimeric monoclonal antibody that binds specifically to the extracellular domain of the human epidermal growth factor receptor (EGFR). Cetuximab is composed of the Fv regions of a murine anti-EGFR antibody with human IgG1 heavy and kappa light chain constant regions and has an approximate molecular weight of 152 kDa. Cetuximab is produced in mammalian (murine myeloma) cell culture [19].

Cetuximab has been approved by FDA as a treatment for EGFR-expressing colon cancer in combination with irinotecan in patients whose disease is refractory to irinotecan-based chemotherapy and as monotherapy for patients with EGFR-expressing colon cancer after failure of both irinotecan and oxaliplatin-based chemotherapy regimens. It is also approved as the first line treatment for in combination with FOLFIRI (irinotecan, 5-fluorouracil, leucovorin) in patients with k-ras mutation-positive colorectal cancer (mCRC) as determined by FDA-approved tests for this use. When used intravenously, clearance is 0.08 to 0.02 L/h/m²; half-life is 112 hours (range 63-230 hours). It is not indicated in patients with k-ras mutation-positive colorectal cancer.

Warnings are serious infusion reactions and cardiopulmonary arrest. The most common adverse events associated with Cetuximab are cutaneous adverse reactions (including rash, pruritus, and nail changes), headache, diarrhea, and infection. The most serious adverse reactions with Cetuximab are infusion reactions, cardiopulmonary arrest, dermatologic toxicity and radiation dermatitis, sepsis, renal failure, interstitial lung disease, and pulmonary embolus.

2. **Bevacizumab**: A recombinant humanized monoclonal antibody directed against the vascular endothelial growth factor (VEGF), a proangiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance
of tumor blood vessels. It has been approved by FDA as first-line therapy for metastatic colorectal cancer in combination with FOLFIRI (5-fluorouracil, leucovorin, irinotecan) or flupyririmidine-oxaliplatin-based chemotherapy, as well as second line treatment of patients with metastatic colorectal cancer (mCRC) whose disease has progressed (i.e., the cancer continues to grow or spread) while on first-line treatment with a non bevacizumab-containing regimen [20].

Warnings are gastrointestinal perforations, surgery and wound healing complications, and hemorrhage. Most common adverse events associated with bevacizumab are epistaxis, headache, hypertension, rhinitis, proteinuria, taste alteration, dry skin, rectal hemorrhage, laceration disorder, back pain and exfoliative dermatitis.

3. Panitumumab: Recombinant human IgG2 kappa monoclonal antibody that binds specifically to the human Epidermal Growth Factor Receptor (EGFR). Overexpression of EGFR is detected in many human cancers, including those of the colon and rectum. When Panitumumab binds to EGFR, it competitively inhibits the binding of ligands for EGFR. This result in inhibition of cell growth, induction of apoptosis, decreased pro-inflammatory cytokine and vascular growth factor production [21]. It has been approved by FDA for the treatment of patients with wild-type KRAS (exon 2 in codons 12 or 13) metastatic colorectal cancer (mCRC) as determined by an FDA-approved test for this use:

- As first-line therapy in combination with FOLFOX
- As monotherapy following disease progression after prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy

Most common adverse events associated with Panitumumab are skin rash with variable presentations, paronychia, fatigue, nausea, and diarrhea.

4. Ramucirumab: It is a recombinant human IgG1 monoclonal antibody that specifically binds to vascular endothelial growth factor receptor 2 (VEGF), and blocks binding of VEGFR ligands, VEGF-A, VEGF-C, and VEGF-D. As a result, ramucirumab inhibits ligand stimulated activation of VEGF Receptor 2, thereby inhibiting ligand-induced proliferation, and migration of human endothelial cells. A pro-angiogenic cytokine. It has been approved by FDA for use in combination with FOLFIRO (folic acid, 5-fluorouracil, and irinotecan) for the treatment of metastatic colorectal cancer in patients whose disease has progressed during or after therapy with bevacizumab. Based on a population PK analysis, the mean (% coefficient of variation [CV%]) volume of distribution at steady state for ramucirumab was 5.5 L (14%), the mean clearance was 0.014 L/hour (30%), and the mean elimination half-life was 15 days (24%). Warnings are gastrointestinal perforations, Hemorrhage, arterial thromboembolic events, Hypertension, surgery and wound healing complications, and Reversible Posterior Leukoencephalopathy Syndrome The most common adverse reactions observed are hypertension, diarrhea, headache, Hyponatremia, anemia and intestinal obstruction [22].

Other MABs that are in various stages of clinical trials are listed in Table 1.

B. Checkpoint inhibitors

There are no checkpoint inhibitors that are currently approved for FDA. However, the few drugs that are in Clinical trials Phase I-III are listed in Table 2.

C. Kinase inhibitors

1. Regorafenib: Regorafenib and its active metabolites inhibit multiple membrane-bound and intracellular kinases, that are involved in normal cellular functions and pathologic processes, including those in the RET, VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-alpha, PDGFR-beta, FGFR1, FGFR2, TIE2, DDR2, Trk2A, Eph2A, RAF-1, RAF, RAF V600E, SAPK2, PTK5, and Abl pathways [30].

Regorafenib has received FDA approval for the treatment of patients with metastatic colorectal cancer (mCRC) who have been previously treated with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, with an anti-VEGF therapy, and, if KRAS wild type, with an anti-EGFR therapy. The coefficient of variation of AUC and Cmax is between 35% and 44%, highly bound (99.5%) to human plasma proteins, metabolized by CYP3A4 and UGT1A9 and elimination half life is 28 hours (14 to 58 hours).

The most common adverse events associated with Regorafenib are asthenia/fatigue, HFSR, diarrhea, decreased appetite/food intake, hypertension, mucositis, dysphonia, and infection, decreased weight, gastrointestinal and abdominal pain, rash, fever, and nausea. The most serious adverse drug reactions in patients receiving Regorafenib are hepatotoxicity, hemorrhage, and gastrointestinal perforation.

Other Kinase inhibitors that are in various stages of clinical trials are listed in Table 3.

D. Vaccine therapy

Tumor antigens that have been targeted in colon cancer include carcinoembryonic antigen (CEA), MUC1, guanylyl cyclase C, and NY-ESO-1. Several clinical studies of cancer vaccines for colon cancer are in ongoing clinical trials.

a. Non FDA approved vaccines (Table 4):

1. FANG vaccine: Autologous tumor cells transfected with a plasmid expressing recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) and bifunctional short hairpin RNA (hi-siRNA) against furin, with potential immunostimulatory and antineoplastic activities.

2. Ad5-hGCCC-PADRE vaccine: A replication-defective,
Table 3. Non-FDA approved kinase inhibitors [31-41].

| Drug               | Clinical trial identifier no. | Phase | Study Design                   | Target               |
|--------------------|-------------------------------|-------|--------------------------------|----------------------|
| Vemurafenib        | NCT02164916                   | Phase II | Randomized, efficacy study, open label | BRAF (V600E) kinase |
| Imatinib           | NCT00041340                   | Phase II | Safety/Efficacy study, open label | TK                  |
| Erlotinib hydrochloride | NCT00032110               | Phase II | Safety/Efficacy study, open label | EGFR                |
| Fraquintinib       | NCT02314819                   | Phase III | Randomized, Efficacy study, Double blind | VEGFRs 1, 2, and 3  |
| Farnitinib         | NCT01762293                   | Phase II | Randomized, Safety/Efficacy study, double blind | RTK                |
| BFIB 1120          | NCT02149108                   | Phase III | Randomized, Safety/Efficacy study, double blind | VEGFR, FGF, PDGFR   |
| Pacritinib         | NCT02277093                   | Phase II | Safety/Efficacy study, open label | JAK2, JAK2V617F     |
| Anlotinib          | NCT02332499                   | Phase II, Phase III | Randomized, Safety/Efficacy study, double blind | RTK                |
| Ruxolitinib        | NCT02119676                   | Phase II | Randomized, double blind         | JAK 1 and 2         |
| MK2206             | NCT01802320                   | Phase II | Efficacy study, open label        | Protein kinase B    |
| ONC201             | NCT02038699                   | Phase I, Phase II | Safety/Efficacy study, open label | Akt/ERK            |

Table 4. Non-FDA approved vaccines [42-46].

| Drug                                | Clinical trial identifier no. | Phase | Study Design                  | Target       |
|-------------------------------------|-------------------------------|-------|--------------------------------|--------------|
| FANG Vaccine                        | NCT01505166                   | Phase II | Randomized, efficacy study, single blind | Tumor cells  |
| Ad5-hGCC-PADRE vaccine              | NCT01972373                   | Phase I | Safety study, open label       | Tumor cells  |
| DEC-205-NY-ESO-1 fusion protein vaccine | NCT01522820               | Phase I | Non-Randomized, safety study, open label | NY-ESO-1     |
| EP2101                              | NCT00054912                   | Phase I | Non-Randomized, safety study, open label | CAP1-6D, PADRE |
| AVX701                              | NCT01890213                   | Phase I | Safety study, open label       | CTL          |

Table 5. Non-FDA approved adoptive Tcell therapy [47].

| Drug                                | Clinical trial identifier no. | Phase | Study Design                  | Target       |
|-------------------------------------|-------------------------------|-------|--------------------------------|--------------|
| Anti-VEGFR2 CAR CD8 plus PBL        | NCT01218867                  | Phase I, Phase II | Non-Randomized, Safety/Efficacy study, open label | VEGFR2      |

recombinant adenoviral serotype 5 (Ad5) encoding human guanylyl cyclase C (hGCC) and the synthetic Pan DR epitope (PADRE), with potential antineoplastic and immunomodulating activities.

3. DEC-205-NY-ESO-1 fusion protein vaccine: A fusion protein, consisting of a fully human monoclonal antibody directed against the endocytic dendritic cell (DC) receptor, DEC-205 linked to the tumor-associated antigen (TAA) NY-ESO-1 with potential immunostimulating and antineoplastic activities.

4. EP2101: A proprietary cancer DNA vaccine that contains multiple natural and modified epitopes derived from the four tumor associated antigens, CEA, HER2/neu, p53, and MAGE 2/3.

5. AVX701: A cancer vaccine, consisting of aliphavirus vector-derived virus-like replicon particles, expressing the 9-amino-acid carcinoembryonic antigen peptide (CAP) 1-6D, with potential antineoplastic activity. Vaccination with this agent may elicit a cytotoxic T lymphocyte (CTL) immune response against CEA-expressing tumor cells.

E. Adoptive cell therapy

In adoptive cell therapy, immune cells are removed from a patient, genetically modified or treated with chemicals to enhance their activity, and then re-introduced into the patient with the goal of improving the immune system’s anti-cancer response.

a. Non FDA approved drugs:

1. Anti-VEGFR2 CAR CD8 plus PBL: A phase I/II trial of anti-VEGFR2 gene engineered CD8+ lymphocytes for colon cancer is ongoing (Table 5).

F. VEGF inhibitor:

1. Aflibercept: Ziv-aflibercept (previously known as Aflibercept) is a recombinant fusion protein, consisting of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2, that are fused to the Fc portion of the human IgG1 immunoglobulin. Ziv-aflibercept injection has been approved by FDA in combination with 5-fluorouracil, leucovorin, irinotecan (FOLFIRI) for the treatment of patients with metastatic colorectal cancer (mCRC), that is resistant to or has progressed following an oxaliplatin-containing regimen [48]. Following 4 mg/kg every two weeks i.v. administration, the elimination half-life was approximately 6 days (range 4-7 days). Steady state concentrations were reached by the second dose. An increased risk of hemorrhage, including severe and sometimes fatal hemorrhagic events has been reported in patients treated with Aflibercept. Patients should be monitored for signs and symptoms of gastrointestinal bleeding and other severe bleeding. Not to be administered to patients with severe hemorrhage. Most common adverse events associated with Aflibercept were leukopenia, diarrhea, neutropenia, proteinuria, AST increased, stomatitis, fatigue, thrombocytopenia, ALT increased, hypertension, weight decreased, decreased appetite, epistaxis, abdominal pain, dysphonia, serum creatinine increased, and headache.

2. BIBF 1120: An orally available cancer cell stemness inhibitor with potential antineoplastic activity. Even though the exact target has yet to be fully elucidated, BIBF1120 appears to target and inhibit multiple pathways involved in cancer cell stemness. This may ultimately inhibit cancer stems cell (CSC) growth, as well as heterogeneous cancer cell growth. CSCs, self-replicating cells that are able to differentiate into heterogeneous cancer cells, appear to be responsible for the malignant growth, recurrence and resistance to conventional chemotherapies (Table 6).
H. mTOR inhibitor

1. PF-05212384: An agent targeting the phosphatidylinositol 3 kinase (PI3K) and mammalian target of rapamycin (mTOR) in the PI3K/mTOR signaling pathway, with potential antineoplastic activity. Upon intravenous administration, PI3K/mTOR kinase inhibitor PKI-587 inhibits both PI3K and mTOR kinases, which may result in apoptosis and growth inhibition of cancer cells overexpressing PI3K/mTOR. Activation of the PI3K/mTOR pathway promotes cell growth, survival, and resistance to chemotherapy and radiotherapy; mTOR, a serine/threonine kinase downstream of PI3K, may also be activated independent of PI3K (Table 7).

1. Cytokine induced killer cell immunotherapy

Cytokine induced killer cells: A preparation of autologous lymphocytes with potential immunopotentiating and antineoplastic activities. Cytokine-induced killer (CIK) cells are CD3- and CD56-positive, non-major histocompatibility complex (MHC)-restricted, natural killer (NK)-like T lymphocytes, generated ex-vivo by incubation of peripheral blood lymphocytes (PBLs) with anti-CD3 monoclonal antibody, interleukin (IL)-2, IL-1, and interferon gamma (IFN-gamma) and then expanded. When reintroduced back to patients after autologous stem cell transplantation, CIK cells may recognize and kill tumor cells associated with minimal residual disease (MRD). CIK cells may have enhanced cytotoxic activity compared to lymphokine-activated killer (LAK) cells (Table 8).

Conclusion

Our success in treating colon cancer is increasing and advancing with the knowledge of the function of the immune system. Immunotherapy has been a promising development in the past few years. The recent activities have increased our understanding of the tumor microenvironment, various immunotherapeutic modalities or combination therapy (like chemotherapy with immunotherapy). However, the effects of such treatment in cancer patients are still in exploratory phase. The complete perspective of immunotherapy treatment has not been realized and/or utilized. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

Table 6. Non-FDA approved cancer stemness inhibitor [49].

| Drug          | Clinical trial identifier no. | Phase | Study Design                      | Target              |
|---------------|-------------------------------|-------|-----------------------------------|---------------------|
| BB860         | NCT01776307                  | Phase II | Non Randomized, Safety/ Efficacy study, open label | Cancer cell stemness |

Table 7. Non-FDA approved PI3 kinase/mTOR inhibitor [50].

| Drug (identifier) | Clinical trial identifier no. | Phase | Study Design                      | Target              |
|------------------|-------------------------------|-------|-----------------------------------|---------------------|
| PF-05212384      | NCT01937715                  | Phase II | Randomized, Safety/ Efficacy study, open label | PI3K/mTOR           |

Table 8. Non-FDA approved cytokine induced killer cells [51,52].

| Drug (identifier) | Clinical trial identifier no. | Phase | Study Design                      | Target              |
|------------------|-------------------------------|-------|-----------------------------------|---------------------|
| Cytokine induced killer cells | NCT02280278 | Phase III | Randomized, Safety/ Efficacy study, open label | Cancer cells |
| Cytokine induced killer cells | NCT01929499 | Phase II | Randomized, Safety/ Efficacy study, open label | Cancer cells |

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