**Bacterial-based therapy: Potential for inflammatory bowel disease**

Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal tract (GIT). The two most prevalent types of IBD are Crohn’s disease (CD) and ulcerative colitis (UC). Symptoms and signs of IBD include abdominal pain, diarrhea, vomiting, anorexia, weight loss, rectal bleeding, edema, ulceration, and loss of mucosal integrity. Some IBD patients may experience extra-intestinal manifestations, such as arthritis, primary sclerosing cholangitis, and uveitis. This article discusses the involvement of bacteria and their role in therapy.

**General etiology/pathogenesis**

Although the exact cause of IBD is unknown, the interplay of genetic factors, immune dysregulation, and infectious agents are thought to have an important role in its pathogenesis.

IBD is not inherited in a classic monogenetic manner; however, some genetic factors are thought to predispose to its development. It was demonstrated that individuals with affected first degree relatives have a higher risk of developing IBD compared to the general population.

Regarding the role of the immune system in IBD pathogenesis, CD is thought to mostly involve a Th1-type mediated response, whereas UC involves a Th2 mediated response. Cytokines produced and released from Th1 cells include Tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and IL-12 however, cytokines released by Th2 include IL-4, IL-5 and IL-13. Other factors that may predispose to IBD development include hygiene, diet and drugs.

**Beneficial role of bacteria**

Physiologically, commensal bacteria have an important role in regulating intestinal development, maintaining intestinal pH and immune homeostasis, and metabolizing a variety of foods and drugs. In addition, some members of the intestinal microbiota, such as *Bacteroides* and *Clostridia* species, produce butyrate and short chain fatty acids (SCFA), which improve the integrity of the epithelial barrier, influence intestinal immune responses, and are sources of energy for colonocytes.

Pattern recognition receptors (PRRs) are a group of receptors with an important function in the recognition of microbes. Examples of PRRs include nucleotide oligomerization domain 2 (NOD2) and toll-like receptors (TLR). Lipopolysaccharide (LPS), an outer bacterial cell wall component, was shown to stimulate the innate immune system through binding to TLR4 and activating the transcription factor nuclear factor kappa B (NF-κB), which is needed to stimulate the production of a variety of cytokines and chemokines needed to mount an immune response. Moreover, commensals play a significant role in the maturation process of the immune response by enhancing the secretion of IgA and IgM during neonatal development. Immunoglobulins can neutralize luminal antigens and carry information on its contents to the mucosal immune system.

**Pathological role of bacteria in IBD**

**Genetic mutations**

Pathologically, bacteria also play a key role in the pathogenesis of IBD. Genetic mutations in bacteria can lead to the expression of toxins and other factors that contribute to the inflammation and damage seen in IBD.

---

*In this issue*

- Bacterial therapy for IBD 1
- Test your knowledge 7
- Topical issues 8
- In the News 13
sis of IBD. One of the well categorized genetic mutations linked to IBD pathogenesis is the mutation in the nucleotide oligomerization domain 2/caspase recruitment domain 15 (NOD2/CARD15) gene, which is found in the cytosol. It has a broad expression profile in the colon, such as in Paneth cells, and it recognizes the bacterial cell wall component muramyl dipeptide (MDP) to activate the key proinflammatory transcription factor, NF-κB. In addition, NOD2 regulates the expression of α-defensin in Paneth cells suggesting a role in regulating a local response in the gut towards pathogenic bacteria.

In normal conditions, defensins, such as α-defensin, assist the immune cells in killing phagocytized bacteria, and are involved in regulating and maintaining the microbial balance in the intestine. It was suggested that IBD patients with a mutation in the NOD2/CARD15 gene have disrupted host ability to localize and eradicate bacteria. This mechanism was linked to a decrease in α-defensin levels in Paneth cells resulting in impaired mucosal tolerance and enhanced intestinal permeability. TLRs are a group of membrane bound and cytosolic PRRs, which are activated by a variety of bacterial ligands. Similar to NOD2/CARD15, their activation will stimulate the downstream signaling of NFκB pathway.

Several reports suggest a physiological role of TLRs in maintaining mucosal and commensal homeostasis and barrier function, through their role in host defense and tissue repair responses to acute injury(1). In IBD TLRs’ expression is modulated leading to inappropriate innate and adaptive immune reactions, and severe inflammation. Moreover, other genes, such as multi-drug resistance 1 (MDR1) gene, IL-10, and transforming growth factor-β (TGF-β), are also thought to be involved in IBD (2).

**Barrier dysfunction**

Mucosal barrier dysfunction is another important factor in IBD pathogenesis. The intestinal epithelium serves as a selective barrier for antigens, limiting their entry from the lumen into the mucosal immune cells.

Epithelial cells are held together by tight junction (TJ) proteins, which limit the passive diffusion of molecules between these cells. TJ proteins include zonula-occludens (ZO), occludins, and claudins. In IBD there is an increase in epithelial permeability, induced by proinflammatory cytokines, such as TNF-α or IFN-γ, or due to increased epithelial cell apoptosis. This leads to bacterial and endotoxins’ translocation; therefore causing persistent activation of the adaptive immunity.

**Pathogenic bacteria**

The intestinal microbiota composition of healthy subjects is different compared to IBD patients. The ratio of protective commensals (Clostridia and Bacteroides) to pathogenic bacteria species is decreased in IBD. In addition, IBD patients have a decreased diversity in intestinal microbiota. The two main bacterial species showing a strong evidence of their role in the pathogenesis of IBD are *E.coli* and *Mycobacterium avium paratuberculosis* (MAP).

**E. coli**

*E.coli* is a predominant aerobic Gram-negative commensal bacterium that helps maintain normal intestinal homeostasis. Increased numbers of *E.coli* were isolated from the mucosa and mesenteric lymph nodes (MLNs) of CD patients(3). In addition, higher levels of antibody titers to *E.coli* was observed in CD patients compared to controls, and 37-55% of patients with CD had increased levels of *E. coli* outer membrane protein C (Omp-C) (4). These species had an increased ability to adhere to gastrointestinal epithelial cells, disrupt the mucosal barrier by α-haemolysin production, and invade and survive inside the epithelial cells. This type of *E.coli* is called adhesive invasive *E.coli* (AIEC), which also stimulates the release of IL-8 and TNF-α, which are two important proinflammatory cytokines playing a role in IBD pathogenesis.

**Mycobacterium avium paratuberculosis**

Another important bacteria involved in IBD pathogenesis is MAP, which is an obligate intracellular
Normally, the GIT commensal bacteria may have either pro- or anti-inflammatory properties. A balanced microbiota is one which is predominantly saccharolytic and contains a large number of Bifidobacteria and Lactobacilli. This balance is usually disturbed in IBD patients. Prebiotics exert their beneficial effects by stimulating the growth of the protective commensal bacteria Bifidobacteria and Lactobacilli, and increasing the resistance to colonization of pathogenic bacteria by secreting antimicrobial compounds, which compete with pathogenic bacteria on the epithelial receptors in the intestine.

Prebiotics have a key role in stimulating the
growth of probiotics as well as other commensals, such as *Eubacteria rectale*. Regarding the role of prebiotics in enhancing the resistance to the colonizion of harmful bacteria, their fermentation results in reduced luminal pH; thus inhibiting the growth of pathogens like *Bacteroides spp*. In addition, they prevent pathogenic bacteria from adhering or colonizing the gut epithelium.

**Effects on barrier function**

Other studies suggest that prebiotics play a role in improving the intestinal barrier function. This was demonstrated in animal models of colitis where prebiotics' administration stimulated the growth of protective bacteria, and enhanced epithelial defense mechanisms against inflammation by stimulating mucin production, which prevents bacterial adhesion and translocation across the epithelial barrier. Dietary fibers which increase mucin production include guar gum and citrus fibers.

**Effects on the immunity**

The balance between pro- and anti-inflammatory cytokines is disturbed in IBD. Prebiotics were found to regulate the production of pro-and anti-inflammatory cytokines thereby reducing the severity of intestinal inflammation. The effects of prebiotics and probiotics, either alone or in combination, on the MLNs and Peyer's patches in rats was examined by Roller et al (7). They found that inulin-enriched oligofructose increased the production of the anti-inflammatory mediator IL-10 in Peyer's patches as well as the secretion of IgA in the ileum. Secretory IgA inhibits pathogenic bacteria from adhering to the intestine and enhances the function of intraperitoneal macrophages.

In addition, in colitis-susceptible HLA-B27 transgenic rats inulin-enriched oligofructose increased intestinal production of anti-inflammatory cytokines, e.g. TGF-β, and reduced expression of pro-inflammatory cytokines, e.g. IL-1β; thus reducing colitis severity (8).

**Effects on SCFA**

As mentioned previously, fermentation of prebiotics results in the production of SCFAs, such as butyrate. Butyrate is a major energy source for colonocytes, and is involved in colonic epithelium maturation and differentiation, mucosal regeneration in the case of atrophy, or apoptosis. In rat MLNs, butyrate inhibits the production of the pro-inflammatory cytokines IFN-γ and IL-2 by inhibiting NF-κB transcriptional factor activity (9).

**Animal models**

The effects of prebiotics were studied in animal models of IBD and in several clinical trials. Dextran sodium sulfate (DSS) is a chemical used to induce colitis in rats by increasing the permeability of the mucosal barrier through its toxic effect on colonocytes. The effects of inulin were studied in this model of colitis (10). After the induction of colitis, inulin-fed rats had a significantly reduced activity of tissue myeloperoxidase (MPO), which is an index of neutrophil infiltration to the colon, with parallel reduction in the mucosal release of inflammatory mediators, such as prostaglandin E2 (PGE2), thromboxane B2 (TXB2), and leukotriene B4 (LTB4), the extent of damaged mucosa, and the severity of crypt destruction.

Further support to the beneficial role of prebiotics in IBD management was also demonstrated in trinitrobenzene sulphonate (TNBS) induced colitis (11).

**Clinical trials**

The effects of prebiotics were also studied in IBD patients, including pouchitis. Pouchitis is an inflammation of the ileo-anal-pouch, which is an internal reservoir placed in the normal location of the rectum after a surgical removal of the large intestine. In one study, it was found that inulin reduced the severity of inflammation of the ileal reservoir mucosa, and this was associated with increased faecal butyrate levels and decreased faecal counts of *Bacteroids*(12). Moreover, the effect of oligofructose-enriched inulin was studied in patients with mild to moderate UC. In this study, faecal concentration of calprotectin was measured to assess intestinal inflammation. Calprotectin is a marker of intestinal inflammation present in granulocytes resistant to metabolic degradation. The level of calprotectin was significantly reduced at day 7 in the group receiving oligofructose-enriched inulin compared to the placebo group. Disease activity scores were also decreased at the end of the study period in the two groups, showing no difference between the prebiotic and placebo group (13).

**Probiotics**

**Mechanism of action**

**Effects on luminal compounds**

Probiotics have a beneficial role in IBD due to their effects on luminal compounds, barrier integrity, and immunity. Probiotics exert an inhibitory effect on the pathogenic bacteria by reducing luminal pH, stimulating the production of bacteriocins, and inhibiting the adhesion of bacteria on epithelial cells. In addi-
tion, intake of probiotics is thought to reduce the space occupied by pathogenic bacteria in the lumen, and compete with pathogens for bacterial substrates.

**Effects on barrier function**

Probiotics enhance the barrier function in several ways. One mechanism is thought to be by increasing the phosphorylation of the TJ proteins actinin and occludin as demonstrated by the administration of *Streptococcus thermophilus* and *Lactobacillus acidophilus* into human intestinal cell lines; thus inhibiting the adhesion and invasion of pathogens (enteroinvasive *E. coli*).

In addition, the probiotic ECN was found to have a significant role in maintaining the function of epithelial TJ by causing redistribution of ZO-2 proteins through the action of protein kinase C signaling pathway. This process prevents the enteropathogenic *E.coli* disruptive effect on T84 epithelial cell monolayers. Moreover, some *Lactobacillus*, e.g. *L. plantarum* 299v, were noticed to induce the expression of intestinal mucin gene in intestinal epithelial HT-29 cell line *in vitro*, thereby preventing the adhesion of enteropathogenic *E.coli*. Moreover, some probiotics interact with TLR2 and TLR4; thereby inducing the production of protective cytokines, such as IL-6 and keratinocyte-derived cytokine-1 (KC-1), which mediate epithelial cell regeneration and the inhibition of cell apoptosis.

**Effects on immunity**

Probiotics have been shown to play a role in modulating the immune system. They can produce anti-inflammatory effects by stimulating secretory IgA, IgM, and IgG production, and enhancing the levels of T-regulatory cells.

Secretory IgA prevents pathogenic bacterial translocation and initiation of inflammatory response. Some probiotics stimulate the production of anti-inflammatory cytokines, such as IL-10 and TGF-β, the secretion of antibacterial substances, and suppress the production of the pro-inflammatory cytokine TNF-α. In addition, PRRs in the intestine recognize microbial associated molecular patterns (MAMPS), such as LPS, peptidoglycans (PG), and demethylated bacterial DNA (CpG DNA). The interaction between MAMPS and PRRs leads to the activation of the innate immune system and production of a protective and/or pro-inflammatory response.

Some probiotics express MAMPS similar to those in the intestine; thus stimulating an innate immune response and directing the adaptive immunity to produce Th1, Th2, or Th3 responses. Th3 cells have an important role in protecting the colonic mucosal surface from nonpathogenic non-self antigens. They inhibit the effects induced by Th1 and Th2 cells. In addition, Th3 was shown to improve the tolerance to bacteria in the gut (14). Figure 1, shows the different postulated mechanisms of action of bacterial-based therapy in reducing colitis severity.

**Animal models**

The use of probiotics has been evaluated in different animal models of colitis with demonstrations of the beneficial effect of VSL#3 in reducing colitis severity through improving barrier function, reducing inflammatory cytokines, and regulating intestinal epithelium cell (IEC) function when administered to IL-10−/− mice (15,16,17).

Another probiotic, ECN, also reduced colitis severity in different models of colitis. Kamada et al studied the role of ECN in both acute and chronic colitis in murine models (18). ECN was found to improve body weight loss and reduce disease activity index as well as macro- and microscopic damage in the acute...
model of colitis. In addition to these findings, they also observed a reduction in the pro-inflammatory cytokines and chemokines levels, such as IFN-γ and MIP-2, in the chronic model of colitis. On the other hand, other studies demonstrated that VSL#3 had no effect on dinitro-benzene sulphonic acid (DNBS) colitis models (19).

Clinical trials
Probiotics may have promising effects on inducing and maintaining remission in UC patients. Their role was investigated in inducing remission in a small number of patients who were non-responsive to conventional therapy, and resulted in a high remission rate (77%) when patients were treated with the probiotic VSL#3 for six weeks (20).

Another study concluded that the combination of VSL#3 and balsalazide had superior effects on inducing remission, and improving health and bowel frequency in UC patients, than either balsalazide or mesalazine alone (21). These favorable effects of probiotics in inducing remission, however, were not observed in patients with CD or pouchitis (22).

Regarding the effects of probiotics on maintaining remission, several studies have been conducted. Malchow et al compared ECN to a placebo in maintaining CD remission for one year (22). The study showed a higher relapse rate in the placebo group, (64%) compared to the probiotic group (33%). Along with their suggested role in inducing remission, probiotics' may have an effect on the maintenance of remission in UC.

Several studies showed equivalent effects of probiotics to 5-ASA in preventing relapse in UC patients. For example, Zocco et al (23) reported similar efficacy of LGG alone or in a combination with mesalazine to mesalazine alone in maintaining remission of UC. However, other studies showed that probiotics may have no benefit or even unfavorable effects in some patient groups. Schultz et al (24) reported no significant differences between LGG and a placebo in maintaining CD remission. In pouchitis patients, Shen et al (25) reported that 81% of patients with antibiotic dependent pouchitis had discontinued VSL#3 because of its adverse effects or due to symptoms recurrence.

Safety profile
As for their safety profile, in theory, probiotics may cause systemic infections, metabolic adverse effects, immune reactions, and gene transfer. In practice, the risk of infection is very low since currently used strains are non-pathogenic. However, a few cases of infections were reported after using L. rhamnosus GG in non-IBD patients. It is important to mention that the majority of those cases occurred in a hospital setting in patients with central venous catheters (14).

Conclusion
IBD is a complex inflammatory disorder of unknown etiology where bacteria are thought to play a significant role in its pathogenesis. The current treatment of this condition involves the use of anti-inflammatory agents, immunosuppressants, and biologics. Bacterial-based therapy (pre-, pro-, and symbiotics) are considered a potential therapy for IBD treatment.

References


Maha Al Harbi
Final Year student, 2011
Faculty of Pharmacy, Kuwait University

TEST YOUR KNOWLEDGE

1. Which of the following drugs is not part of biologic therapy used in the treatment of IBD?

   a) Azathioprine
   b) Infliximab
   c) Adalimumab
   d) Natalizumab
   e) None of the above

2. Which of these is an example of prebiotic?

   a) Oligofructose
   b) Galacto-oligosaccharides
   c) Lactulose
   d) Inulin
   e) All of the above

3. Regarding their safety profile, in theory, which of the following effects is not produced by probiotics?

   a) Systemic infections
   b) Metabolic adverse effects
   c) Constipation
   d) Immune reactions
   e) Gene transfer

Is there a problem?
A 45 y old patient was prescribed the drug given in the prescription, the dose of which was increased for his high cholesterol level. Is there any major error in the prescription?

TYM HOSPITAL

<table>
<thead>
<tr>
<th>Patient Name: Mr. Khaled</th>
<th>Age: 45 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address: Street No. 11</td>
<td></td>
</tr>
<tr>
<td>Rx</td>
<td>Atorvastatin 100mg tablet</td>
</tr>
<tr>
<td></td>
<td>Once a day x 1 month</td>
</tr>
<tr>
<td>Dr. RXC</td>
<td>Signature</td>
</tr>
<tr>
<td></td>
<td>Date: 20/02/12</td>
</tr>
</tbody>
</table>

Answer (Prescription Exercise)
Dose is wrong. It’s overdose. The maximum dose for atorvastatin is 80mg once daily.

(Source: British National Formulary, Version 62, page 164)
Cytisine, for smoking cessation

Cytisine, is an original Bulgarian drug from the plant Cytisus laborinum L (Golden rain acacia). All parts of the plant contain the alkaloid cytisine, the greatest amount (up to 3%) is contained in the seeds.

Cytisine, has been available in some Eastern European and former Soviet countries, such as Russia, for more than 40 years. It is used as an aid to smoking cessation under the brand name Tabex. However, the drug has not previously been tested in a way that would meet modern regulatory standards in UK and other countries.

To test the effectiveness of the drug, a study was done using 740 volunteers who were either given the drug or placebo for 25 days. (West et al. New Engl J Med 2011; 365:1193) in a randomised, double-blind trial. Individuals who smoked 10 or more cigarettes per day, and who were willing to attempt to stop smoking permanently were enrolled in the study. They were randomised to receive either cytisine or a placebo pill (370 in each group). The participants took cytisine or the placebo for 25 days, and were then assessed 6 and 12 months after the treatment period had ended, to determine whether they had managed to give up smoking or if they had relapsed. The participants agreed prior to the trial not to take any other medications to stop smoking. Both groups received a minimal amount of counselling during the study.

Results after 12 months showed that 8.4% of the participants randomised to receive cytisine had not relapsed (in other words, had successfully quit smoking), compared to 2.4% of the participants randomised to receive placebo. This was a difference of 6%, which equated to people taking cytisine being 3.4 times more likely to give up than those taking a placebo. The researchers report that this increase in rate of giving up smoking is higher than that reported for the existing drug varenicline (smokers taking varenicline are 2.3 times more likely to quit than those taking a placebo) and nicotine-replacement therapy (1.6 times more likely). Gastrointestinal (stomach and intestine) side effects, predominantly stomach-ache, dry mouth, dyspepsia and nausea, were reported significantly more frequently in participants receiving cytisine (13.8%) than those receiving placebo (8.1%). There were no other side effects, which were significantly more frequent in the group receiving cytisine. The two groups experienced similar rates of drug discontinuation and dose reduction.

Although this study only lasted 12 months and was not large enough for an assessment of uncommon adverse events, the researchers report that the latest Periodic Safety Update Report provided to the European Authorities, based on more than 7 million exposed persons, did not identify any safety signals: in other words, the drug is considered safe. In addition, the lower price of cytisine, as compared with that of other pharmacotheapies for smoking cessation, make it an affordable treatment to advance smoking cessation globally.

It can be concluded that in this promising 12-month trial (involving a treatment period of 25 days), participants taking cytisine were more than three times more likely to give up. Although individuals in the group receiving cytisine experienced more gastrointestinal side-effects, the researchers said that other uncommon side effects are unlikely as this drug has been available in other countries for more than 40 years.

However, the trial was not large enough to assess the uncommon adverse events that could occur with the drug. Because the drug is in the same class as others linked to neuropsychiatric side effects and suicidal ideas the researchers recommend continued surveillance of the 7 million people reported to be taking it.

In this study, the participants were given minimal behavioural support, such as counselling. The researchers suggest that combining cytisine with more intensive behavioural support could potentially increase the absolute quit rates. Given that the trial was relatively small and short it is likely that more research will be needed to confirm its effectiveness and safety before regulators can approve its use.
Big business with drug repositioning: finding new uses for existing drugs

Drug repositioning or re-purposing - an effort to find new uses for existing drugs - is not a new idea. Viagra, for example, was first tested as a hypertension treatment before it became a blockbuster drug for erectile dysfunction. Thalidomide, an anti-nausea medication for pregnant women that was pulled from international markets in the 1960s after it was found to cause terrible birth defects, got US FDA approval for its use in bone-marrow cancer.

By simply identifying a different disease that can be treated with an existing drug, companies can skip preclinical and early clinical trials, and thus leapfrog much of the estimated 10–15 years, and the more than $1 billion it takes to bring a new drug to market. Companies developing new drug candidates can also recoup major losses if a drug fails in Phase II or III trials by finding a new indication in which to move it forward.

But in the past, drug repositioning has been an unpredictable process - an occasional happy accident when a doctor noticed a strange side effect or a researcher documented an off-label use for a drug. Biovista already has two candidates for treating progressive multiple sclerosis, both of which proved successful in animal models of the disease, and the company has established partnerships with Pfizer and the FDA. It is one of a handful of data-savvy young biotechs working to transform drug repositioning from an occasional coincidence to a systematic pursuit of new markets.

NuMedii, a California-based biotech started in 2008, is one of the youngest companies to join the repositioning movement. The company’s technology maps gene activity patterns from a database containing molecular profiles of over 300 diseases. If two diseases share a molecular profile - a similar set of activated genes - perhaps they could also share drugs. Drugs that work for heart attack patients, for instance, should perhaps be tested for effectiveness in people with muscular dystrophy, as the two conditions share similar activated pathways.

Drug repositioning offers advantages of reduced time and risk as several phases common to de novo drug discovery and development can be bypassed because reposition candidates have frequently been through several phases of development for their original indication; candidates have frequently been through several phases of development for their original indication.
Such predictions at Stanford are being validated with unpublished but promising results in animal models, testing two existing medications that may be repurposed to treat Crohn’s disease and lung cancer, respectively. The patents for both drugs have long since expired, which turns out to be a double-edged sword. While the drugs are both cheap and available to repurpose as new treatments for two serious illnesses, it is challenging to find a pharmaceutical partner to fund the Phase II trials required to get the drugs approved for these new uses.

Though the FDA offers patent protection for repurposed drugs in their new indication, there’s no guarantee that a doctor won’t prescribe a generic version that came on the market after the original patent expired, even though it’s technically not approved for the new use. Still, the experiments demonstrate the technology’s promise in predicting which diseases an existing drug might treat -a critical proof of concept that the technology could prove valuable when applied to patented drugs that aren’t selling well in their current indication.

Biovista is also eager to partner with pharmaceutical companies to reposition marketed drugs, as well as those that have failed at some step during preclinical or clinical development. The company’s technology collects publicly available data on diseases, drugs, targets, and adverse events, then organizes the data into 20 comparable categories -such as gene associations and comorbidities - and scans for similarities. They can navigate all 23,000 diseases and all 6,000 adverse events against all 20,000 human targets and 95,000 drugs and pharmacologically active compounds with reasonable data in the public domain. The technology’s predictions are then tested in vitro and in vivo, with an impressive 70 % success rate. The platform’s prognostic value has caught the attention of Pfizer, who last November inked a deal with Biovista to identify novel indications for a number of existing Pfizer medications.

Biovista and NuMedii take a bioinformatics-heavy approach. Melior Discovery, a Pennsylvania-based biotech has relied on “systematic serendipity” to identify potential new uses for old drugs, a.k.a. the stumble-upon-them technique. Using a non-hypothesis-driven method, the company runs drugs through a series of 40 animal models representing a wide gamut of illnesses, from Alzheimer’s disease to asthma to overactive bladders, looking to see what works. It’s a way to uncover potential therapeutic effects that otherwise would not have been predicted. As haphazard as the approach sounds, it has produced remarkable results. In 5 years, the company has run over 250 compounds through the 40 animal models and found potential therapeutic uses about 30 percent of the time. Their lead candidate, MLR-1023, a kinase activator discarded as a treatment for gastric ulcers after poor Phase II results, showed activity in a mouse model of Type II diabetes and will soon begin a Phase II trial for the disease. In 2007, Pfizer established an Indications Discovery Unit in St. Louis, a group dedicated to repositioning the company’s failed compounds and finding new indications for the promising ones. GlaxoSmithKline has shown a similar interest, but is exploring bioinformatics methods to prove that drug repositioning is a profitable effort before committing significant resources.

Source: http://www.the-scientist.com/article/display/58079/#ixzz1KVKT1Inp
Immunotherapy in melanoma

The incidence and mortality rate of cutaneous melanoma have been increasing more rapidly than any other cancer over the last 3 decades. Melanoma accounts for 1-3% of all malignant tumors, and its incidence is increasing by 6-7% each year. Melanoma that is detected early can be surgically resected, resulting in a 5-year patient survival rate of more than 80%. However, once melanoma has metastasized, it is almost always fatal; the 5-year patient survival rate is less than 5% and patients die within 6-9 months.

Currently, there are several approved treatments for metastatic melanoma, including chemotherapy and biologic therapy as both single treatments and in combination, but none is associated with a significant increase in survival.

The chemotherapeutic agent dacarbazine is the standard treatment for metastatic melanoma, with a response rate of 15-20%, although most responses are not sustained. Dacarbazine, a non-classical alkylating agent, is the most widely used chemotherapeutic agent and has been approved by the US FDA for the treatment of melanoma. The anti-tumor activities of dacarbazine result in growth arrest and cell death through nucleic acid methylation or direct DNA damage.

One of the main problems with melanoma treatment is chemotherapeutic resistance. Following treatment with dacarbazine, melanoma cells activate the extracellular signal-regulated kinase pathway, which results in over-expression and secretion of interleukin (IL)-8 and vascular endothelial growth factor. Melanoma cells utilize this mechanism to escape from the cytotoxic effect of the drug. IL-8 and VEGF may act as autocrine and paracrine factors on tumor and endothelial cells, promoting growth and metastasis of melanoma. IL-8 can serve as a survival factor or as an angiogenic factor acting on endothelial cells. Since dacarbazine treatment causes upregulation of IL-8, which is an important mediator of melanoma tumor growth, angiogenesis, and metastasis, it is considered as a potential target for immunotherapies against human melanoma.

The development of fully human neutralizing antibodies against IL-8 (anti-IL-8-monoconal-antibody [ABX-IL8]) has been reported. ABX-IL8 is a neutralizing antibody that binds IL-8 and blocks its ability to bind to its receptor. ABX-IL8 also inhibits migration, degradation, and activation of neutrophils. In vitro, a combination of dacarbazine and anti-IL-8 antibodies caused a decrease in cell viability in metastatic melanoma cell lines. In preclinical studies, ABX-IL8 inhibited tumor growth, angiogenesis, and metastasis of human melanoma in vivo. The combination treatment with dacarbazine and ABX-IL8 may potentiate the cytotoxic effect of the drug.

Furthermore, formation of metastasis is a multistep process that includes melanoma cell adhesion to endothelial cells. The adhesion molecule MUC18 is up-regulated in metastatic melanoma and its expression correlates with increased tumor thickness and metastatic potential. MUC18 is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily and functions as a calcium-independent adhesion molecule that interacts with an unknown heterophilic ligand.

Expression of MUC18 in human melanoma cell lines directly correlates with the ability of the cells to metastasize in vivo. MUC18 is strongly expressed on metastatic melanoma cells and less frequently expressed on nevus cells. MUC18 is also expressed on endothelial cells, enabling heterotypic adhesion between melanoma cells and endothelial cells, which could indicate that MUC18 is involved in promoting invasation and extravasation.

A fully human antibody against MUC18 (anti-MUC18 monoclonal antibody [ABX-MA1]) has been developed and it has shown promising results in preclinical studies. Administration of ABX-MA1 was shown to result in a decrease in tumor growth and metastasis after injection of highly metastatic melanoma cell lines. ABX-MA1-treated tumors also exhibited a decrease in angiogenesis. In vitro, ABX-MA1 caused disruption of spheroid formation, a decrease in transcription and activity of MMP-2, and a decrease in invasion through Matrigel™-coated filters. Moreover, MUC18 antibodies have also been shown to inhibit the attachment of melanoma cells to HUVEC and the tube-like formation of HUVEC-cells, suggesting that this approach may target not only the tumor but also the tumor microenvironment.

Therefore, anti-MUC18 antibodies may be administered alone or in combination with chemotherapy to improve treatment of metastatic melanoma. Since ABX-MA1 acts at a different phase of the
metastatic cascade than ABX-IL8, combination therapy using both antibodies given as a 'cocktail' should also be considered. Therefore, by targeting multiple pathways and utilizing these antibodies in combination with the standard chemotherapeutic agent dacarbazine, survival for patients with metastatic melanoma will significantly improve.


Barriers to widespread clinical application of genotyping

The US FDA recommends that doctors genotype patients before prescribing more than 70 commonly-used medications for specific genetic biomarkers. These tests, the agency suggests, can help physicians identify those in which the drug is less efficacious, poorly metabolised, or dangerous. But medicine is still far from a day when drugs and treatment regimens are fitted precisely to a patient’s genomic profile.

According to a 2008 survey conducted by the American Medical Association (AMA) and Medco Research Institute, even though 98% of physicians agreed that the genetic profiles of their patients may influence drug therapy, only 10% believed they were adequately informed about how to test their patients for biomarkers that may predict the safety and/or efficacy of a particular drug.

Indeed, while new biomarkers are identified every day, and researchers are continuing to collect more and more information about genetic variants that confer some amount of disease risk or predict a specific response to a treatment, that information has yet to be widely implemented in the clinic.

The AMA states on its website that physicians today can use more than 1,200 genetic tests for more than 1,000 different diseases to help diagnose and treat their patients, but only 13% of the 10,000 doctors who responded to the survey had ordered a genetic test for a patient in the preceding 6 months. But while physicians have generally been slow to adopt the practice of screening patients to search for genetic information of relevance to drug treatments, (pharmacogenomics) neither research nor regulation has stalled, as evidenced by the FDA’s re-labeling of dozens of approved drugs with biomarkers that affect their safety or efficacy in specific patient population.

Pharmacogenomics is probably an area where personalized medicine is really able to deliver because those are tests that can be clearly associated with a particular therapy. In some cases, testing patients for the labeled pharmacogenomic markers has become critical. For example, the FDA strongly recommends that doctors prescribing the HIV drug abacavir test their patients for HLA-B*5701 allele. Individuals carrying that allele who take abacavir could become hypersensitive to the drug, which can lead to a systemic, potentially fatal flu-like illness. A study published in the New Engl J Med (2008: 358 (6):568) found that testing for the presence of HLA-B*5701 in HIV patients taking abacavir eliminated hypersensitivity reactions. Abacavir is a black and white example which shows that if you don’t do genetic testing, you’re omitting something that’s clearly a standard of care today.

Other pharmacogenomic biomarkers, while helpful, aren’t as obvious. Studies have yielded mixed results, for example, about whether genetic testing for different CYP2C19 alleles in patients taking the
anticoagulant drug Plavix can indicate proper dosing schedules to improve how the drug is metabolised.

Similarly, identifying single nucleotide polymorphisms in two genes, *CYP2C9* and *VKORC1*, in patients taking another blood thinner, warfarin, can help guide optimum dosing to prevent over anticoagulation, but the markers’ predictive ability varies widely across races, according to a 2008 study (*Pharmacogenomics* 9(5):511-26).

Still, recent results suggest that genotyping patients who are receiving warfarin can improve health outcomes. A 2010 nationwide study that compared the effectiveness of warfarin among different patient populations, conducted by Medco and the Mayo Clinic, found that patients receiving the drug who had been genotyped to determine their *CYP2C9* and *VKORC1* status were hospitalised about 30% less than patients whose genotypes were unknown. Remarkably, only a handful of physicians out of the thousands contacted for the study were even aware that a genetic test existed that could potentially improve warfarin dosing in patients (*J Am Coll Cardiol* 2010; 55(25):2804-12).

This lack of implementation is one reason why personalised medicine is not yet a widespread clinical reality - a barrier that is called by some experts as the “adoption gap” between advances in the lab and benefits in the clinic. The world is awash in biomarker content and it is essential to find the most effective mechanism to drive awareness among the primary care physician base.

Part of the problem, is that physicians underestimate the predictive power of genetic risk factors for certain diseases or treatment outcomes. If doctors are made aware of the fact that certain genetic biomarkers can be just as powerful as traditional predictors, they may be more inclined to use them to help personalise treatment regimes.

There’s a problem with clinical uptake of new genomic tools and biomarkers, adding that researchers also need to do a better job of demonstrating the clinical utility of such advances. Furthermore, with the sheer volume of new genomic information coming out of labs across the globe, it’s difficult for physicians to stay abreast of the latest advances that could improve the way they treat their patients.

According to Felix Frueh, president and head of genomics initiatives at Medco Research, the ‘build it and they will come’ approach to personalized medicine is not going to work. If one is not actively reaching out to the people who are practicing, nobody is going to come.

**References**

2. www.newsweek.com/2010/05/21/let-there-be-life.html


### Safety & effectiveness of Acetadote for Acetaminophen toxicity

Acetaminophen (APAP) is one of the most frequent medications in both accidental and intentional overdoses, with over 48,000 exposures treated in U.S. health care facilities in 2005. APAP is now recognised as the leading cause of acute liver failure in hospitalised patients.

Until 2004, treatment consisted of either oral N-acetylcysteine (NAC) or filtered oral NAC administered intravenously (i.v.). Both i.v. and oral NAC, however, have demonstrated similar efficacy in treating APAP toxicity. However, oral NAC administration is often associated with several difficulties.

In 2004, the FDA approved i.v. acetylcysteine (Acetadote®), a sterile, pyrogen-free solution. To date, there have been few reported post-marketing data. Although an effective oral formulation of NAC already exists, more institutions are looking to use i.v. acetylcysteine due to
the ease of administration and lack of noxious effects.

Buckley et al.\(^4\) evaluated the clinical presentations and outcomes of patients treated with i.v. acetylcysteine for APAP toxicity. They performed a retrospective chart review of patients treated with i.v. acetylcysteine for APAP ingestion. The primary outcome measures were: adverse reactions to and effectiveness of i.v. acetylcysteine, as defined by elevation of transaminases, liver failure, renal failure, death, and hospital length of stay (LOS). Data collected included: comorbidities, allergies, intentionality, timing and dosing of i.v. acetylcysteine, hospital LOS, transaminases > 1000 IU/L, development of liver failure requiring transplant, development of renal failure requiring hemodialysis, death, and anaphylactoid reactions. Overall, 25% patients developed transaminases > 1000 IU/L, 6% of them died and 3% received liver transplants. Of the patients treated within 8 h, none died or developed liver or renal failure, and only 1 developed transient transaminase elevation > 1000 IU/L.

Not only did the early administration of i.v. acetylcysteine for APAP toxicity result in a favorable outcome, but it also resulted in a decrease in LOS as well. When treated within 8 h of ingestion, there was an absolute reduction of 2 days in median hospital LOS. In the patients treated outside of 8 h, the median LOS was 3 days, whereas the group treated within 8 h had a median LOS of only 1 day. This finding suggests that administration of i.v. acetylcysteine within the recommended 8 h may result in the saving of significant hospital resources.

It is well established that early administration of NAC results in replenished stores of glutathione, resulting in decreased risk of hepatotoxicity secondary to the toxic APAP metabolite, N-acetyl-p-benzoquinoneimine.\(^5,6\) In this study, despite the fact that only 23% of patients received i.v. acetylcysteine within the recommended 8 h, the overall outcome of those treated was favorable. Of the patients who died or required a liver transplant, all were treated with i.v. acetylcysteine beyond 8 h from the time of ingestion. It is unclear why initiation of i.v. acetylcysteine therapy was delayed in such a large percentage of the study population. The most likely explanations are: unclear history, late presentation, and delayed diagnosis.

There has been a hesitancy to use i.v. formulations of NAC due to a reported increased risk of anaphylactoid reactions, especially during infusion of the loading dose.\(^7\) (Since the FDA approval of i.v. acetylcysteine has changed to recommend a 60-min initial infusion, instead of 15 min, to decrease the risk of anaphylactoid reactions). 9% patients developed anaphylactoid reactions, 2 of whom received the i.v. acetylcysteine bolus over 15 min. Five of these patients were treated pharmacologically and completed treatment, and one had treatment discontinued for undocumented reasons.

In conclusion, intravenous acetylcysteine seemed to be a safe and effective formulation of N-acetylcysteine. Furthermore, the data support i.v. acetylcysteine administration over 60 min and within 8 h to achieve all of the following: low risk of anaphylactoid reaction, favorable outcome, and shorter hospital length of stay.

References
Drug headlines of 2011

Developing new medicines is a tricky business, requiring sound science, regulatory savvy, and marketing skills. The past year has seen success and failure in all these areas. This article highlights a few of the noteworthy drug developments of 2011.

First new lupus drug in 52 years

After more than 18 years of development, the FDA approved the first drug to treat lupus in more than a half-century. Benlysta (belimumab) is a human monoclonal antibody, produced by Human Genome Sciences and GlaxoSmithKline, that inhibits B-cells proliferation, a proposed mechanism underlying the autoimmune disorder. The once-monthly injectable drug has limited efficacy, reducing the symptoms of 43% of patients compared to 34% of those on placebo in a Phase III trial, but nonetheless for a disease with few approved treatments, this is still a reasonable advance.

Hope for hepatitis

People with hepatitis C had cause for celebration with the approval of two new drugs for the liver-infecting virus. In May, the FDA approved Incivek (telaprevir) from Vertex Pharmaceuticals and Merck’s Victrelis (boceprevir).

Incivek is used for patients who have either not received interferon-based drug therapy for their infection or who have not responded adequately to prior therapies. Incivek is approved for use with interferon therapy made up of peginterferon alfa and ribavirin. The current standard of care for patients with chronic hepatitis C infection is peginterferon alfa and ribavirin taken for 48 weeks. Less than 50% of patients respond to this therapy.

In three phase 3 clinical trials with 2,250 adult patients who were previously untreated, or who had received prior therapy, 79% of those receiving Incivek experienced a sustained virologic response, i.e. the infection was no longer detected in the blood 24 weeks after stopping treatment, which suggests that hepatitis C condition has been cured. This is 20-45% higher than current standard of care.

Incivek is a pill taken three times a day with food. It should be taken for the first 12 weeks in combination with peginterferon alfa and ribavirin. Most people with a good early response to the combination regimen can be treated for 24 weeks rather than the recommended 48 weeks of treatment with the standard of care. Incivek and also Merck’s Victrelis (boceprevir) which also received FDA approval are both protease inhibitors, which bind to the virus and preventing it from multiplying.

New cancer drugs: gene-ie in a bottle

The FDA approved two mutation-specific cancer drugs in August alongside diagnostic tests for those mutations. Genentech’s Zelboraf (vemurafenib) for late-stage melanoma targets a specific mutation in the B-RAF oncogene. It is being approved with a first-of-a-kind test called the cobas 4800 BRAF V600 Mutation Test and is marketed with a companion diagnostic for the mutation from Roche.

Zelboraf was reviewed under the FDA’s priority review program that provides for an expedited six-month review of drugs that may offer major advances in treatment or that provide a treatment when no adequate therapy exists. Zelboraf and the companion BRAF V600E test were approved ahead of the drug’s and the companion diagnostics’ goal dates of October/November 2011.

Zelboraf’s safety and effectiveness were established in a single international trial of 675 patients with late-stage melanoma with the BRAF V600E mutation who had not received prior therapy. Patients were assigned to receive either Zelboraf or dacarbazine, another anti-cancer therapy. The trial was designed to measure overall survival. The median survival of patients receiving Zelboraf has not been reached (77% still living) while the median survival for those who received dacarbazine was 8 months (64% still living).

The approval of Zelboraf and the cobas test is a great example of how companion diagnostics can be developed and used to ensure patients are exposed to
highly effective, more personalized therapies in a safe manner. The FDA’s approval of the cobas 4800 BRAF V600 Mutation Test was based on data from the clinical study that also evaluated the safety and effectiveness of Zelboraf. Samples of a patient’s melanoma tissue were collected to test for the mutation.

In March, FDA had approved Yervoy (ipilimumab), another new treatment for late-stage melanoma that also showed patients live longer after receiving the drug.

In late August the FDA approved Pfizer’s Xalkori (crizotinib) to treat certain patients with late-stage (locally advanced or metastatic), non-small cell lung cancers (NSCLC) who express the abnormal anaplastic lymphoma kinase (ALK) gene.

This ALK gene abnormality causes cancer development and growth. About 1-7% of those with NSCLC have the ALK gene abnormality. Patients with this form of lung cancer are typically non-smokers.

Xalkori was approved with a companion diagnostic test that will help determine if a patient has the abnormal ALK gene, a first-of-a-kind genetic test called the Vysis ALK Break Apart FISH Probe Kit. It is the second such targeted therapy approved by the FDA in 2011. The approval of Xalkori with a specific test allows the selection of patients who are more likely to respond to the drug. It works by blocking certain kinases, including the protein produced by the abnormal ALK gene.

Xalkori’s safety and effectiveness were established in two multi-center, single-arm studies enrolling a total of 255 patients with late-stage ALK-positive NSCLC. A sample of a patient’s lung cancer tissue was collected and tested for the ALK gene abnormality prior to study enrollment. The studies were designed to measure objective response rate, the % of patients who experienced complete or partial cancer shrinkage. Most patients in the studies had received prior chemotherapy.

In one study, the objective response rate was 50 % with a median response duration of 42 weeks. In another, the objective response rate was 61% with a median response duration of 48 weeks. The FDA based its approval of the Vysis ALK Break Apart FISH Probe Kit on data from one of these studies.

Xalkori is being approved under the FDA’s accelerated approval program, which allows the agency to approve a drug to treat a serious disease based on clinical data showing that the drug has an effect on an endpoint that is reasonably likely to predict a clinical benefit to patients. The program is designed to provide patients with earlier access to promising new drugs, followed by further studies to confirm the drug’s clinical benefit.

These drugs represent a new paradigm for drug development, where a small but well-defined fraction of people get a very well-defined drug.


Correct answers: 1-a; 2-e; 3-c