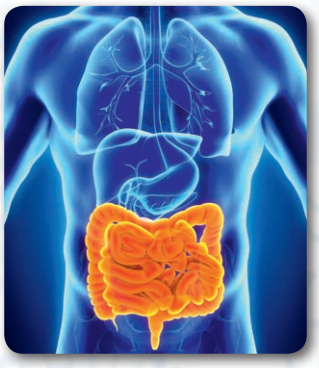
BGs AS HUMAN VACCINES

Bacterial Ghosts (BGs) are empty, non-living bacterial particles generated from pathogenic or nonpathogenic Gram-negative bacteria through a proprietary, genetically induced inactivation process. The bacteria leading to BGs can be genetically manipulated in various ways to enrich them with target antigens essential for other infectious diseases in their cell envelope. Thus, BG vaccines against infectious diseases can be generated directly from the pathogen, if it is a Gram-negative bacterium, or through production of BGs carrying antigens from pathogens of interest. Latter pathogens can be either bacteria, viruses, or eukaryotes – i.e. fungi, protozoa or worms.

BG Vaccines against Infectious Diseases

Based on its BG vaccine technology platform BIRD-C is pursuing the following activities in the field of human vaccines



Enteric Diseases

Sexually Transmitted Diseases

Respiratory Diseases

Parasitic Diseases

Competitive Advantages of BG Vaccines

- Retention of natural surface components and full immunogenic potential of living bacteria
- A conservation of immunogenicity of recombinant antigens introduced into BGs
- No need to add adjuvants to elicit strong humoral and cellular immune responses
- High stability and immunogenicity of freeze-dried product for several years at ambient temperature
- Stable in liquid formulation stored at +4°C for minimum of 1 year
- BGs can be used as carriers of DNA vaccines
- BG platform offers an easy, rapid and one-step production process for subunit vaccines

BGs represent an innovative vaccination approach with competitive advantages over existing human vaccines

BIRD-C's commercial model is to develop human vaccines against various infectious diseases and to license the vaccine(s) to pharmaceutical companies.

A list of *in vivo* proof of concept studies for the induction of immune responses for enteric diseases, sexually transmitted diseases, respiratory and ear infections and parasitic diseases are given on back side of this leaflet.

Overview of immune responses against BGs for Human applications

General Group	Disease caused	BG carrier	Immune response/conferred protection
Enteric diseases	Cholera	*Vibrio cholerae*	• Vibrio specific humoral immune response in mice vaccinated intraduodenally with *V. cholerae* BGs. • Protective humoral response and cross-serogroup protective immunity in rabbits, vaccinated s.c. or i.m. with *V. cholerae* BGs. • Cross-protective humoral response along with dose dependent protective immunity against intraduodenal challenge in rabbits vaccinated orally with *V. cholerae* BGs.
	Gastritis, Dyspepsia, Gastric ulcer, Cancer	*Helicobacter pylori*	• Significant reduction of bacterial load. 15/20 of orally vaccinated mice were protected without use of adjuvant; complete protection after co-administration of *H. pylori* BGs with mucosal adjuvants.
		Salmonella typhimurium	• *S. typhimurium* BGs carrying *H. pylori* outer inflammatory protein gene A (oipA) encoded DNA vaccine elicited mixed Th1/Th2 immune response and reduced bacterial colonization in vaccinated mice.
	Bloody diarrhea	*Escherichia coli*	• Ag-specific humoral immune response. Increased survival time of the intraperitoneally or s.c. vaccinated mice using *E. coli* BGs • T-Cell stimulation in rabbits vaccinated s.c. with *E. coli* BGs • EHEC BGs given orally to mice showed 93.3% protection, whereas single rectal immunization conferred full protection to mice against 50% lethal heterologous EHEC challenge.
	Shigellosis	*Shigella flexneri*	• Strong serum anti-CS3 IgG titers observed in mice vaccinated intranasally with *S. flexneri* BGs carrying ETEC CS3 pili when compared to formalin killed vaccine.
	Hepatitis B	*Escherichia coli*	• Significant HBcAg-149 specific humoral immune response in mice vaccinated with *E. coli* BGs carrying OmpA-HBcAg-149 in IM and on OM.
	Typhoid like disease	*Salmonella typhimurium*	• Significantly increased survival of mice vaccinated with *S. typhimurium* BGs.
Sexually transmitted diseases	Acquired immune deficiency syndrome (AIDS)	*Escherichia coli / Vibrio cholerae*	• Cellular immune response against IM anchored HIV-1 gp41 protein in mice. • Humoral immune response against IM anchored HIV-1 RT in mice.
	Chlamydiosis/ Genital infections	*Vibrio cholerae*	• Local mucosal and systemic Th1 type specific immune response against IM anchored rMOMP/OMP2 protein of *C. trachomatis* in mouse. • Mucosal, systemic humoral and Th1 type specific immune response and cross-protection against heterologous chlamydia serovars in mice vaccinated with *rVCG* carrying IM anchored CTA2B protein of *C. trachomatis*. • Antigen specific mucosal and systemic Th1 responses; mice immunized with rVCG expressing chlamydial MOMP and HSV-2 glycoprotein D elicited secretory IgA and IgG2a antibodies to both antigens.
Respiratory diseases and Middle ear infection	Chronic obstructive pulmonary disease	*Mannheimia haemolytica*	• Mice vaccinated intradermal/i.m. with *M. haemolytica* BGs elicited β-galactosidase humoral and cellular specific immune responses.
	Respiratory tract infection/Middle ear infection	*Escherichia coli*	• NTHi Omp26-specific humoral immune response in rats vaccinated mucosally with *E. coli* BGs carrying recombinant SbSA-OMP26 (cytoplasm) or OMP26 (in periplasmic space) .
Parasitic disease	Leishmaniasis	*Vibrio cholerae*	• Significant humoral and cellular Th1 immune response against IM anchored rGP63 surface protein of leishmania in vaccinated mice.

Ab: Antibody; Ag: Antigen; BG: Bacterial Ghost; CS3: coli surface antigen 3; CTA2B: Cholera toxin A2/B subunits; EHEC: enterohaemorrhagic *E. coli*; ETEC: enterotoxigenic *E. coli*; HBcAg: Hepatitis B core antigen; HIV-1 gp41: Human immunodeficiency virus type 1 glycoprotein 41; HIV-1 RT: Human immunodeficiency virus type 1 reverse transcriptase; HSV-2: Herpes simplex virus type 2; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgG2a: Immunoglobulin G subclass 2a; IM: Inner membrane; i.m.: intra muscular; MOMP: Major outer membrane protein; NTHi: Nontypable *Haemophilus influenzae*; OM: Outer membrane; OMP: Outer membrane protein; rGP63: recombinant glycoprotein 63; rVCG: Recombinant *Vibrio cholera* Ghosts; s.c.: subcutaneous; Sbs: Surface-layer protein of *Bacillus stearothermophilus*; Th1:T helper cell type 1; Th2: T helper cell type 2;