intrinsically resistant tumors and in others that acquire resistance during chemotherapy treatment. In fact, when the *mdr* gene that encodes Pgp is transfected into drug-sensitive cells, they became resistant.[9] Generalizations about the structural features required for a compound to be a Pgp inhibitor are hampered by the very heterogeneous chemical structure of compounds that have shown this property. The extensive list of traditional Pgp substrates includes the anthracyclines (doxorubicin and daunorubicin), vinca alkaloids (vinblastine and vincristine), colchicine, epipodophyllotoxins (etoposide and teniposide), and paclitaxel. In addition to these compounds, some of the modern antitumor drugs, such as the antileukemia drug imatinib, the marine natural product trabectedin, and the calicheamicin conjugate gemtuzumab ozogamicin, are excreted by this mechanism.

The functionally related protein MRP1 confers resistance to the vinca alkaloids, anthracyclines and epidophyllotoxins, as well as glucuronide, glutathione, and sulfate conjugates of drugs. Other members of the ABC family, such as MRP2 and MRP3, transport the same drugs, whereas MRP4 and MRP5 transport nucleotide and nucleoside analogs. The breast cancer resistance protein (BCRP) is a member of the ABCG subfamily that partially overlaps the substrate specificity of Pgp and MRPs, conferring resistance to mitoxantrone, methotrexate, topotecan, and SN-38.[10]

The ABC transporters are asymmetrically distributed when they are present in the same cell. For instance, in intestinal epithelium cells, Pgp, MRP2, and BCRP are located at the brush borders, whereas MRP1 and MRP3 are located at the basolateral surface. They are also differently expressed in normal conditions. Thus, Pgp is expressed in several organs, such as intestine, lung, kidney, liver, adrenal gland, certain hematological cells, blood–brain barrier, and placenta, which suggests that it is important in limiting the oral absorption of xenobiotics and contributes to limit access to the central nervous system through the blood–brain barrier. Several of these roles have been confirmed in knockout mice. Most MDR modulators act by binding to Pgp, inhibiting its drug-effluxing activity, and some act by indirect mechanisms, including inhibition of the expression of the *mdr1* gene.[11]

Because Pgp and MRPs are membrane-bound proteins, their study by nuclear magnetic resonance or X-ray diffraction techniques is difficult. To date, there is no detailed information on their structures and their substrate or inhibitor binding sites, which prevents proper *de novo* design approaches, but crystallization of prokaryotic ABC membrane proteins has helped in the design of Pgp inhibitors. The crystal structure of mouse Pgp, which has 87% sequence identity to human Pgp, was described in 2009,[12] and that of *Caenorhabditis elegans* was disclosed in 2012.[13]

## 2.2 INHIBITION OF P-GLYCOPROTEIN

Human Pgp is formed by 1280 amino acids and has two homologous halves, each containing a transmembrane domain with six α-helices (TM1–6 on TMD1 and TM7–12 on TMD2) and a hydrophilic nucleotide-binding domain (NBD1 and NBD2) that is located at the cytoplasmatic face of the membrane. Pgp is glycosylated at the first extracellular loop and phosphorylated by protein kinase C, which respectively affect its integration in the membrane and its transport function. Pgp may be modulated by competitive inhibitors that directly interact with TMDs or NBDs[14] or by noncompetitive inhibitors that interact with an allosteric residue relevant for its activity.

ABC proteins, and Pgp in particular, recognize a wide spectrum of compounds, but they all have in common a relatively high lipophillicity—a feature that has led to the proposal of two models to explain how this protein works. In the *hydrophobic vacuum cleaner* model, drugs partitioning into the membrane spontaneously translocate to the cytoplasmic leaflet and gain access to the Pgp substrate-binding