Bile acids are synthesized from cholesterol in hepatocytes. After their biosynthesis, bile acids undergo enterohepatic circulation mediated by both hepatocyte and enterocyte transporters (18). In the hepatocyte, several members of the human ATP-binding cassette (ABC) superfamily have been identified as bile acid transporters, including the bile acid (salt) export pump ABCB11 (BSEP), which is the major canalicular bile salt transporter in hepatocytes (4, 24), and the multidrug resistance protein ABCC4 (MRP4), which functions as a bile acid transporter (16). The cycling of bile acids across the sinusoidal membrane (19, 29) is mediated by both hepatocyte and enterocyte transporters, including the bile acid (salt) export pump ABCB11 (BSEP), which is the major canalicular bile salt transporter in hepatocytes (4, 24), and the multidrug resistance protein ABCC4 (MRP4), which functions as a bile acid efflux pump at the sinusoidal membrane of human hepatocytes (29). Although ABCC3 (MRP3) also is localized to the sinusoidal membrane (16), it has been reported to be a relatively poor transporter for bile acids (1, 37). In addition to the export pumps, several transporters in the sinusoidal membrane of hepatocytes mediate the uptake of bile acids (9), including the Na⁺-taurocholate cotransporting polypeptide NTCP (SLC10A1) and the sodium-independent organic anion transporting polypeptides OATP1B1 (OATP-C/OATP2) and OATP1B3 (OATP8) (18).

Hepatocytes are the major source of reduced glutathione (GSH) in plasma. As a physiological antioxidant, GSH is involved in many biochemical processes and serves as the major transport form of cysteine (5, 23). Therefore, GSH needs to be transported across the sinusoidal membrane of the hepatocyte to enable GSH delivery to other tissues (2). Three members of the human multidrug resistance protein family (ABCC/MRP) have been identified as GSH export pumps. The first is ABCB1 (MRP1; Refs. 20, 22), which is not expressed in hepatocytes in detectable amounts (14). The second is ABCC2 (MRP2), a low-affinity GSH transporter (25), which is localized to the canalicular membrane of hepatocytes (14). The third is ABCC4, which is localized to the sinusoidal membrane of hepatocytes and has been shown to function as a cotransporter for GSH and the conjugated bile acid cholytaurine (C-tau, taurocholate) (29). In the coeflux by ABCC1 and ABCC4, GSH can be replaced by several GSH analogs, including S-methyl-glutathione and ophthalmate (22, 29).

ABCC4 has the lowest number of amino acids of the MRP family and transports a broad range of structurally diverse substances, including sulfated steroids as well as several drugs (17, 35). It is widely distributed in human epithelial cells, and in most of them, ABCC4 is localized to the basolateral membrane (19, 29, 30). An exception is the renal proximal tubular cell, in which ABCC4 has an apical localization (33). The ABCC4-mediated transport was potently inhibited by a widely used inhibitor of ATP-dependent ABC transporters, the quinoline derivative MK571, as well as by known ABCC4 substrates, such as dehydroepiandrosterone 3-sulfate, methotrexate, and folate (29).

The localization of ABCC4 to the sinusoidal membrane of human hepatocytes, together with the identification of C-tau and GSH as cosubstrates for ABCC4, suggest important functions of this export pump in the physiology and pathophysiology of the liver (29). We previously proposed that ABCC4 mediates the cycling of bile acids across the sinusoidal membrane and along the sinusoid (29). The cycling of bile acids may act to regulate the intrahepatocyte concentration of bile acids, whereas the efflux of GSH serves as a source of GSH for other tissues. Bile acid efflux also could be of major importance in case of impaired bile acid transport into bile.
The aim of the present study was to define the substrate specificity of ABCC4 toward common natural conjugated and unconjugated bile acids, as well as to quantify the stoichiometry of bile acid-GSH cotransport.

MATERIALS AND METHODS

Materials. [22,23-3H]deoxycholate (DC-gly; 1.1 TBq/mmol), [22,23-3H]cholate (CDC-gly; 0.4 TBq/mmol), [22,23-3H]cholyltaurine (CDC-tau; 0.4 TBq/mmol), [22,23-3H]ursodeoxycholate (UDC; 0.4 TBq/mmol), [22,23-3H]ursodeoxycholate (UDC-tau; 0.4 TBq/mmol), [22,23-3H]cholate (0.9 TBq/mmol), [22,23-3H]deoxycholate (1.1 TBq/mmol), [22,23-3H]ursodeoxycholate (UDCA; 0.4 TBq/mmol), and [22,23-3H]deoxycholate (1.1 TBq/mmol) were synthesized at the University of California, San Diego as described previously (32). [3H(G)]cholesterylglucuronide (CDC-gly; 0.4 TBq/mmol), [22,23-3H]ursodeoxycholate (UDC; 0.4 TBq/mmol), and [glycine-2-3H]glutathione (GSH; 1.9 TBq/mmol) were obtained from PerkinElmer Life Science (Boston, MA). Glycine-1-3H]cholylglycine (C-gly; 2.1 GBq/mmol) was obtained from American Radio-labeled Chemicals (St. Louis, MO). Unlabeled bile acids, GSH, acivicin, and diethiothreitol (DTT) were obtained from Sigma (St. Louis, MO).

Cell culture and cell lines. Chinese hamster lung fibroblasts V79 cells permanently expressing high levels of recombinant human ABCC4 (V79-ABCC4) were grown in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% (vol/vol) fetal bovine serum and 100 U/ml penicillin-streptomycin and kept at 37°C and 5% CO2.

Preparation of membrane vesicles. Outside-out membrane vesicles from transfected V79 cells were prepared as described previously (13). In brief, the cells were lysed by incubation in hypotonic buffer (0.1 mM EDTA, 0.5 mM sodium phosphate, pH 7.0) for 1.5 h, followed by homogenization with a Potter-Elvehjem homogenizer. After centrifugation of the homogenate at 12,000 g (10 min at 4°C), the postnuclear supernatant was centrifuged at 100,000 g for 45 min at 4°C. The resulting pellet was suspended in incubation buffer (250 mM sucrose, 10 mM Tris-HCl, pH 7.4), homogenized with a tight-fitting Dounce (glass/glass) homogenizer, and layered over 38% (wt/vol) glycerol, and stored in liquid nitrogen.

 Vesicle transport studies. ATP-dependent transport of 3H-labeled bile acids into membrane vesicles from transfected V79 cells. The ATP-dependent accumulation of 3H-labeled bile acid at the standard concentration of 5 μM in membrane vesicles from ABCC4- and vector-transfected cells was negligible in the absence of GSH compared with the presence of GSH for all tested bile acids (Fig. 1, B, D, and F). The presence of 5 mM GSH caused an increase of the ATP-dependent 3H-labeled bile acid accumulation in membrane vesicles from ABCC4-transfected cells (Fig. 1, A, C, and E and Fig. 2B). Although membrane vesicles from vector-transfected cells also showed an ATP-dependent bile acid transport in the presence of GSH, transport mediated by the ABCC4-containing membrane vesicles was at least twofold higher, as shown in Table 1. No stimulation was observed in the presence of 1 mM DTT (not shown). Transport by the control membrane vesicles was consistent with the presence of endogenous hamster Abcc4 in the V79 cells. Although hamster Abcc4 in V79 cells could not be detected at the protein level by currently available antibodies, the presence of hamster Abcc4 mRNA in V79 cells was detected with RT-PCR using Abcc4-specific primers (not shown).

 Vesicles from ABCC4-transfected V79 cells transported the following bile acids in a GSH-dependent manner as summarized in Table 1: for DC-gly, the rate was 31.6 pmol·mg protein−1·min−1 (Fig. 1C); for CDC-gly, 35.7 pmol·mg protein−1·min−1 (Fig. 1E); and for CDC-tau, 51.9 pmol·mg protein−1·min−1. For UDC-gly, the rate was 33.8 pmol·mg protein−1·min−1, and for UDC-tau, 36.8 pmol·mg protein−1·min−1 (Fig. 2A). For trihydroxy bile acids, the rate for C-gly was 19.4 pmol·mg protein−1·min−1, and that for C-tau was 47.1 pmol·mg protein−1·min−1. The unconjugated bile acid cholate was transported at a rate of 8.9 pmol·mg protein−1·min−1 (Fig. 1A). However, no transport could be measured for the four glycoform bile acids, the rate for CDC-gly was 35.7 pmol·mg protein−1·min−1, and that for C-tau was 47.1 pmol·mg protein−1·min−1. The unconjugated bile acid cholate was transported at a rate of 8.9 pmol·mg protein−1·min−1 (Fig. 1A). However, no transport could be
detected for the unconjugated dihydroxy bile acids UDCA and deoxycholate in either ABCC4-containing vesicles or control vesicles under our experimental conditions. Transport rates of vesicles from vector-transfected V79 cells for the corresponding bile acids in the presence of GSH were much smaller, as summarized in Table 1.

Kinetic analysis of ABCC4-mediated ATP-dependent bile acid transport in the presence of glutathione. To further characterize the affinity of ABCC4 for the different bile acids, we determined $K_m$ and $V_{max}$ values in ABCC4-containing vesicles for each bile acid in a concentration range from 2 to 50 $\mu$M and in the presence of 5 mM GSH (Table 2). CDC-tau was the bile acid with the lowest $K_m$ value of 3.6 $\mu$M, followed by CDC-gly with a $K_m$ value of 5.9 $\mu$M. DC-gly and C-tau showed $K_m$ values of 6.7 and 7.7 $\mu$M, respectively. The taurine and glycine conjugates of UDCA exhibited different $K_m$ values, with higher affinity of ABCC4 for UDC-tau (7.8 $\mu$M) than for UDC-gly (12.5 $\mu$M; Table 2 and Fig. 2C). The lowest affinity was observed for cholate (14.8 $\mu$M) and C-gly (25.8 $\mu$M; Table 2). Because membrane vesicles with similar transport rates for all bile acids, and thus similar $V_{max}$ values, were used, the transport efficiency could be calculated for each bile acid (Table 2). The ratio $V_{max}/K_m$ obtained for the various bile acids resulted in the following ranking from high to low transport efficiency: CDC-tau > C-tau > DC-gly > UDC-tau > CDC-gly > UDC-gly > C-gly > cholate (Table 2).

Ursodeoxycholate conjugate-mediated inhibition and its kinetic characterization. ATP-dependent transport of the unconjugated bile acid UDCA into membrane vesicles from transfected cells was not detectable under our experimental conditions. However, ABCC4-mediated ATP-dependent transport of its taurine and glycine conjugates was observed in the presence of 5 mM GSH as noted above. Because UDCA is used widely in clinical practice to treat cholestatic liver disease, and because its dominant metabolites in humans are its glycine and taurine conjugates, we tested whether UDCA as well as UDC-
Bile acid transport in the presence of 5 mM GSH was calculated from the ATP-dependent transport of \( [3H] \) GSH (Fig. 2), 12.9 pmol/mg protein for UDC-gly and UDC-tau, at a standard concentration of 20 \( \mu \)M caused a competitive inhibition of the C-gly-GSH cotransport with \( K_i \) values of 56.2 \( \mu \)M for UDCA (Fig. 2), 12.9 \( \mu \)M for UDC-gly (Fig. 2D), and 10 \( \mu \)M for UDC-tau (not shown).

Table 1. Bile acid transport in the presence of 5 mM GSH in ABCC4-containing vesicles and in control vesicles

<table>
<thead>
<tr>
<th>Bile acid + 5 mM GSH</th>
<th>Transport in ABCC4-Containing Vesicles, pmol/mg protein ( -1 ) min (^{-1} )</th>
<th>Transport in Control Vesicles, pmol/mg protein ( -1 ) min (^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate</td>
<td>8.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Cholyglycine</td>
<td>19.4</td>
<td>10.3</td>
</tr>
<tr>
<td>Cholyltaurine</td>
<td>47.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Deoxycholylglycine</td>
<td>31.6</td>
<td>13.4</td>
</tr>
<tr>
<td>Chenodeoxycholylglycine</td>
<td>35.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Chenodeoxycholyltaurine</td>
<td>51.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Ursodeoxycholylglycine</td>
<td>33.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Ursodeoxycholyltaurine</td>
<td>36.8</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Bile acid transport in the presence of 5 mM GSH was calculated from the ATP-dependent bile acid transport obtained between 1 and 10 min in ABCC4-containing vesicles and in control vesicles obtained from hamster V79 cells. Bile acid transport in control vesicles is likely due to endogenous hamster Abcc4 detected by RT-PCR. Data represent mean (SD) values from a triplicate determination reproduced independently at least once.

Double-labeling experiments for measuring simultaneous ATP-dependent transport of \( [3H] \) glutathione and \( [14C] \) labeled bile acids into vesicles from transfected V79 cells. To demonstrate the cotransport of GSH and bile acids, we measured the simultaneous ATP-dependent transport of 5 mM \( [3H] \) GSH with 5 \( \mu \)M \( [14C] \) C-tau or that of 5 mM \( [3H] \) GSH with different concentrations of \( [14C] \) C-gly (Fig. 3). High background of the

Table 2. Kinetic constants for ABCC4-mediated bile acid cotransport in the presence of 5 mM GSH

<table>
<thead>
<tr>
<th>Bile Acid + 5 mM GSH</th>
<th>( K_m ), ( \mu )M</th>
<th>( V_{max} ), pmol/min ( -1 ) mg protein ( -1 )</th>
<th>( V_{max}/K_m ), pmol/min ( -1 ) mg protein ( -1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate</td>
<td>14.8 ± 0.8</td>
<td>75 ± 15</td>
<td>5.1</td>
</tr>
<tr>
<td>Cholyglycine</td>
<td>25.8 ± 0.8</td>
<td>175 ± 37</td>
<td>6.8</td>
</tr>
<tr>
<td>Cholyltaurine</td>
<td>7.7 ± 0.3</td>
<td>154 ± 10</td>
<td>20</td>
</tr>
<tr>
<td>Deoxycholylglycine</td>
<td>6.7 ± 0.6</td>
<td>116 ± 16</td>
<td>17.3</td>
</tr>
<tr>
<td>Chenodeoxycholylglycine</td>
<td>5.9 ± 0.3</td>
<td>93 ± 8</td>
<td>15.8</td>
</tr>
<tr>
<td>Chenodeoxycholyltaurine</td>
<td>3.6 ± 0.1</td>
<td>83 ± 2</td>
<td>23.1</td>
</tr>
<tr>
<td>Ursodeoxycholylglycine</td>
<td>12.5 ± 0.5</td>
<td>130 ± 15</td>
<td>10.4</td>
</tr>
<tr>
<td>Ursodeoxycholyltaurine</td>
<td>7.8 ± 0.6</td>
<td>133 ± 19</td>
<td>17.1</td>
</tr>
</tbody>
</table>

V79-ABCC4 membrane vesicles from the same membrane preparation were used for kinetic analysis of several bile acids together with 5 mM GSH. The \( K_m \) values were calculated from double-reciprocal plots. Data represent mean (SD) values from 3 determinations each performed in duplicate.
GSH transport into vesicles from vector-transfected cells was obtained, which is probably due to other endogenous GSH efflux mechanisms present in all cells (23).

The following transport rates were obtained in a 20-min time period for 5 mM GSH and 5 μM C-gly in double-labeling experiments: for GSH transport, 122.9 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 41.4 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (Fig. 3A); and for C-gly transport, 7.1 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 3.4 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (Fig. 3B). From these transport rates, ABCC4-mediated transport rates were subsequently obtained: 81.5 pmol·mg protein⁻¹·min⁻¹ for GSH and 3.7 pmol·mg protein⁻¹·min⁻¹ for C-gly, resulting in an approximate C-gly-to-GSH ratio of 1:22. When double-labeling experiments were performed at 5 mM GSH and 10 μM C-gly, the approximate C-gly-to-GSH ratio was maintained at 1:19 when the ABCC4-mediated transport was measured within the first 20 min: 157.6 pmol·mg protein⁻¹·min⁻¹ for GSH and 8.2 pmol·mg protein⁻¹·min⁻¹ for C-gly. At higher C-gly concentrations, the relaative amounts of GSH and C-gly coefflux were not constant; however, much more GSH than C-gly was consistently cotransported.

When C-gly was replaced with C-tau, the cotransport with GSH was corroborated by the following transport rates: for GSH transport, 110.2 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 9.7 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells; and for C-tau transport, 10.3 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 5.5 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (not shown). The resulting ABCC4-mediated C-tau-to-GSH ratio was 1:21. Under the same conditions, ATP-dependent transport of 5 mM [³H]GSH in the absence of bile acids into membrane vesicles from vector- and ABCC4-transfected V79 cells was detected with similar transport rates: 3.2 pmol·mg protein⁻¹·min⁻¹ for vector-transfected cells and 2.0 pmol·mg protein⁻¹·min⁻¹ for ABCC4-transfected cells (not shown).

**DISCUSSION**

In the present study, we have examined in detail the bile acid specificity for GSH-bile acid cotransport by human ABCC4, an export pump localized to the sinusoidal membrane of human hepatocytes. Transport studies in inside-out membrane vesicles demonstrated ATP-dependent transport into ABCC4-containing membrane vesicles for the glycine- and taurine-conjugated bile acids predominating in human biliary bile acids, as well as for the conjugates of UDCA and the unconjugated bile acid cholate in the presence of physiological GSH concentrations (Figs. 1 and 2, Table 1). Whether other known ABCC4 substrates influence GSH transport or whether their transport is modulated by GSH is currently unknown.

The $K_m$ values obtained for all bile acids in the presence of GSH were in the micromolar range and thus in the range of physiological concentrations (Table 2). The highest affinity was observed for CDC-tau with a $K_m$ value of 3.6 μM and the lowest affinity for C-gly with a $K_m$ value of 25.8 μM (Table 2). UDC-gly and UDC-tau, the amidated conjugates of UDCA, showed $K_m$ values of 7.8 and 12.5 μM (Fig. 2C), respectively, which are in the range of $K_m$ values obtained for most of the naturally occurring bile acids in humans (Table 2). CDC-tau followed by C-tau had the highest transport efficiency in ABCC4-containing membrane vesicles (Table 2). Transport affinities of all bile acids were comparable and almost identical to those obtained for the canalicular bile salt export pump ABCB11 (4, 24) with the difference that ABCC4-mediated bile acid transport is a GSH-dependent transport process. Thus the kinetic analysis reveals that ABCC4 is able, at least in principle, to compete with ABCB11 for bile acids in hepatocytes.

UDCA is widely used for the treatment of cholestatic liver diseases (26). In the hepatocyte, UDCA is rapidly conjugated with glycine and taurine. These conjugates may protect the liver against hepatotoxicity of endogenous bile acids by a variety of mechanisms (3). Although UDCA is present in human bile only in trace amounts, treatment with UDCA increases its concentration in plasma and bile, and UDCA becomes the predominant circulating bile acid (3). Although our data indicate that ABCC4 mediates ATP-dependent transport of UDC-tau and UDC-gly (Fig. 2, A and C), transport of labeled UDCA could not be demonstrated using membrane vesicles for reasons that need to be defined in future studies. However, UDCA was able to compete for the ABCC4-mediated ATP-dependent transport of C-gly (Fig. 2D), suggesting that UDCA also may be a substrate for the ABCC4-mediated GSH cotransport. Thus our results indicate that ABCC4 can...
Bile acids can then reenter into more portally situated hepato-
cytocytes should mediate active efflux of GSH and bile acids into
lar ABCB11 mediates the secretion of bile acids into bile (Fig.

For C-tau as well as for C-gly at concentrations between 5
and 10 μM and a constant concentration of 5 mM GSH, the
bile acid-to-GSH ratio was no longer constant, but the amounts
of bile acid transported were invariably greater than the amounts
of bile acid transported. Thus this transport process can be
described as an obligatory coefflux of GSH and bile acids
without a strict stoichiometry. Other cotransporters have been
described with stoichiometries differing from 1:1, such as the
human Na⁺-glucose cotransporter SGLT1, an apical membrane
protein, which couples the transport of 2 Na⁺ ions and 1
molecule with ~210 water molecules (34).

Under physiological conditions in the hepatocyte, canalicu-
lar ABCB11 mediates the secretion of bile acids into bile (Fig.

Fig. 4. Scheme of bile acid transport processes of the hepatocyte. Bile acids in
hepatocytes are transported into bile by the bile salt export pump ABCB11
(BSEP). After enterohepatic circulation through the intestine, bile acids are
taken up into the hepatocyte by the Na⁺-taurocholate cotransporting polypept-
id NTCP (SLC10A1) and/or the organic anion transporters OATP1B1
(OATP-C/OATP2) and OATP1B3 (OATP8). ABCC4, which is localized to
the sinusoidal membrane of hepatocytes (29), mediates high-affinity cotrans-
port of GSH and bile acids into sinusoidal blood and thus competes for bile
acid transport into bile via ABCB11. From sinusoidal blood, bile acids can
then reenter into more portally located hepatocytes by the uptake transporters
described.

argued that biliary excretion and sinusoidal efflux of a com-
 pound may occur at the same time because of the involvement
of different intracellular compartments. Thus the study by
Proost et al. supports our proposed model of bile acid perco-
lation along the sinusoid by ABCC4 when at the same time bile
acids are excreted into bile by ABCB11. The requirement of
GSH for the bile acid transport by ABCC4, but not by
ABCB11, may be part of a regulatory mechanism to control
intracellular concentrations of bile acids. In addition, hepatic
GSH is predominantly released across the basolateral mem-
brane of hepatocytes into the blood circulation as the major
source of plasma GSH, cysteine, and cystine. Thus ABCC4
can be considered as a new pathway for the efflux of GSH into
the systemic circulation, as pointed out in our earlier publication
(29).

Under cholestatic conditions, the concentration of sub-
stances, which are normally excreted into bile, may increase
intrahepatically and cause hepatotoxicity. One compensatory
mechanism that prevents hepatotoxicity is the efflux of sub-
stances into blood across the sinusoidal membrane, as demon-
strated for leukotriene metabolites formed in the hepatocytes
of Mrp2-deficient mutant rats (8, 15) and presumably for bile
acids that are excreted in urine in cholestatic liver disease.

The mechanisms that preserve hepatocytes from bile acid
accumulation have been investigated in cholestatic animal
models (6, 31). Mice lacking the farnesoid X receptor/bile acid
receptor have a reduced expression of Abcb11 but an increased
expression of Abcc4 mRNA as well as increased plasma
concentrations and urinary excretion of bile acids (31). A
similar adaptive response with increased Abcc4 protein expres-
sion has been observed in obstructive cholestasis in rats (6).
However, species differences must be taken into account. A recent study in patients with progressive familial intrahepatic cholestasis, who have elevated serum concentrations of bile acids, showed a marked upregulation of ABCB4 protein and ABCC4 mRNA in BSEP-deficient patients suffering from mutations in the ABCB11 gene, as well as in ABCB4 (MDR3)-deficient patients with mutations in the ABCB4 (MDR3) gene (12). On the other hand, protein expression of ABC3, which has been proposed to mediate bile acid transport (36), was unaltered in liver samples from these cholestatic patients (12). Comparison of the affinities of both transporters, ABC3 and ABC4, for bile acids indicates that ABC4 is the better sinusoidal bile acid export pump compared with ABC3. Thus ABC4 appears to be a major player in buffering the intracel-
sinusoidal bile acid export pump compared with ABC3. Thus ABC4, for bile acids indicates that ABC4 is the better

ACKNOWLEDGMENTS

We thank Anne T. Nies for many helpful discussions and critical reading of the manuscript.

GRANTS

This work was supported in part by the Cooperation Program in Cancer Research of the Deutsches Krebsforschungszentrum (DKFZ) and Israel’s

REFERENCES


