Lung resistance-related protein (LRP) predicts favorable therapeutic outcome in Acute Myeloid Leukemia

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There is conflicting evidence that MDR1, MRP2 and LRP expression is responsible for chemotherapy resistance. We conducted this study to explore their role in AML therapy outcomes. Bone marrow and peripheral blood samples of 90 AML patients, receiving chemotherapy, were analyzed by real time PCR. Gene expression was calculated by the $2^{-\Delta\Delta C_t}$ method. The patients who had a persistent remission were labelled ‘Good Responder’ (GRes) whereas, those with relapse or drug resistance were labelled ‘Poor Responders’ (PRes). Higher LRP expression in bone marrow, but not in peripheral blood, was positively associated with persistent remission ($p = 0.001$), GRes ($p = 0.002$), 1-year overall as well as disease-free survival ($p = 0.02$ and $p = 0.007$, respectively). Marrow and blood MDR1 and MRP2 expression did not differ significantly between the above groups. Logistic regression analysis showed that only a diagnosis of acute promyelocytic leukemia (APL; M3) or high marrow LRP expression significantly predicted a favorable therapeutic outcome. This is the first report showing that high bone marrow LRP expression predicts significant favorable therapeutic outcome. Peripheral blood LRP expression as well as marrow and blood MDR1 and MRP2 expression have no predictive value in AML patients treated with standard dose cytarabine and daunorubicin 3+7 regimen.

Successful chemotherapeutic treatment in acute myeloid leukemia (AML) remains a challenge as a substantial number of patients do not achieve complete remission (CR) and many of those who do respond relapse later. Although drug resistance has remained a point of focus for many researchers, a lot more still needs to be explored. Since the presence of a drug inside target cells is imperative for successful treatment, the role of efflux transporters, such as ATP-binding cassette (ABC) transporters, is also implicated.

One of the ABC transporter family member, ABCB1, also called multidrug resistance protein 1 (MDR1) or permeability-glycoprotein (P-gp), is involved in cellular efflux of xenobiotics, including chemotherapeutic agents. Researchers have focused on MDR1 expression in many drug resistant hematological and solid cancers, yielding inconsistent results.

Another ABC transporter, ABCC2, also called multidrug resistance-associated protein 2 (MRP2), (formerly known as canalicular multispecific organic anion transporter - cMOAT) is commonly found on hepatocyte canalliculi, intestines and kidney cells, and transports various chemicals including drugs. Like MDR1, overexpression of MRP2 has also been related to chemo-resistance.

A third protein is lung resistance-related protein (LRP), also known as major vault protein (MVP or VAULT1). LRP is described as a drug efflux transporter and has been accredited to impart chemo-resistance. Although the function of LRP is still not fully understood, its role in the formation of barrel-shaped vault organelles is recognized. Vaults transport different molecules between nucleus and cytoplasm. In addition to MVP, vaults contain vault poly-ADP-ribose polymerase (vPARP), telomerase-associated protein 1 (TEP1) and vault RNA (vRNA). vPARP identifies DNA damage and adds PAR so that the DNA damage is tagged for repair, while TEP1 is involved in telomere formation. LRP is normally expressed in bone marrow. Positive or higher expression has been associated with adverse outcomes in leukemia as well as multiple solid tumors. In this study we explored the association of gene expression of MDR1, MRP2 and LRP with clinical outcomes of AML chemotherapy.

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Results

Baseline characteristics are given in Table 1. Most of the patients were between 15–40 years, and the most predominant type was “AML with maturation” (48.9%). Myeloperoxidase (MPO) was tested to establish myeloid lineage in 76 patients, of which 62 were positive. Patient data for FLT3, NPM1, PML-RARα, MLL mutation and karyotyping was available only for a limited number of patients (Table 1). 56 patients (62%) achieved CR after first induction, however 19 (34% of CR; 21% of total) relapsed later. Resistant and relapsed patients were collectively labelled as 'poor responders' (PRes) (58.9%), while patients with persistent remission (41.1%) were labeled 'good responders' (GRes).

| Parameters               | N  | Percent |
|--------------------------|----|---------|
| Age groups               |    |         |
| <15 Years                | 3  | 3.3     |
| 15–40 Years              | 62 | 68.9    |
| 41–60 Years              | 24 | 26.7    |
| >60 Years                | 1  | 1.1     |
| Gender                   |    |         |
| Male                     | 66 | 73.3    |
| Female                   | 24 | 26.7    |
| AML classification (who) |    |         |
| APL (M3) with t 15:17    | 17 | 18.9    |
| AML without maturation (M1)| 15 | 16.7 |
| AML with maturation (M2) | 44 | 48.9    |
| Others:                  |    |         |
| - Translocation 6:9      | 2  | 2.2     |
| - AML with minimal differentiation (M0) | 2 | 2.2 |
| - Acute Myelomonocytic Leukemia (M4) | 2 | 2.2 |
| - Acute Panmyelosis with fibrosis | 1 | 1.1 |
| - Myeloid proliferations related to Down syndrome | 1 | 1.1 |
| Unknown                  | 6  | 6.7     |
| MPO status               |    |         |
| Negative                 | 14 | 15.6    |
| Positive                 | 62 | 68.9    |
| Unknown                  | 14 | 15.6    |
| FLT3 mutation            |    |         |
| Negative                 | 35 | 38.9    |
| Positive                 | 7  | 7.8     |
| Unknown                  | 48 | 53.3    |
| NPM1 mutation            |    |         |
| Negative                 | 13 | 14.4    |
| Unknown                  | 77 | 85.6    |
| PML-RAR mutation         |    |         |
| Negative                 | 4  | 4.4     |
| Positive                 | 5  | 5.6     |
| Unknown                  | 81 | 90.0    |
| MLL mutation             |    |         |
| Negative                 | 10 | 11.1    |
| Positive                 | 5  | 5.6     |
| Unknown                  | 75 | 83.3    |
| Karyotyping              |    |         |
| Unfavorable              | 18 | 20.0    |
| Favorable (APL)          | 7  | 7.8     |
| Normal                   | 24 | 26.7    |
| Unknown                  | 41 | 45.6    |
| Therapeutic response     |    |         |
| Resistant                | 34 | 37.8    |
| Relapse                  | 19 | 21.1    |
| Persistent Remission     | 37 | 41.1    |
| Survival status          |    |         |
| Died                     | 42 | 46.7    |
| Alive                    | 44 | 48.9    |
| Unknown                  | 4  | 4.4     |
| Final outcome            |    |         |
| Poor (Resistant + Relapse)| 53 | 58.9    |
| Good (Persistent Remission)| 37 | 41.1   |

Table 1. Baseline Characteristics of the Study Population (AML patients, N = 90).
Table 2. Median expression values (and inter-quartile ranges) of MDR-1, MRP-2 and LRP among study population.

| Parameters                  | Bone Marrow Med 25th | 75th | | | Blood Med 25th | 75th | | | |
|-----------------------------|----------------------|------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| AML Classification          |                      |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| APL (M3); t(15;17)          | 17                   | 0.06 | 0.01 | 0.11 | 0.15 | 0.00 | 0.68 | 3.23 | 0.34 | 15.70 | 14              | 0.06 | 0.03 | 0.20 | 0.02 | 0.00 | 0.13 | 0.71 | 0.29 | 3.94 |
| AML without maturation (M1) | 14                   | 0.03 | 0.00 | 0.35 | 0.06 | 0.01 | 0.28 | 0.59 | 0.30 | 2.42 | 13              | 0.07 | 0.03 | 0.86 | 0.33 | 0.01 | 1.45 | 1.73 | 0.37 | 4.43 |
| AML with maturation (M2)    | 40                   | 0.06 | 0.00 | 0.14 | 0.01 | 0.00 | 0.06 | 0.78 | 0.33 | 4.25 | 38              | 0.12 | 0.00 | 0.25 | 0.03 | 0.00 | 0.11 | 1.68 | 0.67 | 3.69 |
| Others                      | 11                   | 0.00 | 0.00 | 0.05 | 0.01 | 0.00 | 0.07 | 1.04 | 0.29 | 9.90 | 12              | 0.37 | 0.02 | 0.92 | 0.21 | 0.00 | 16.50 | 1.22 | 0.29 | 31.80 |
| AML Classification (Prognostic) |                  |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| All Others                  | 61                   | 0.04 | 0.00 | 0.13 | 0.01 | 0.00 | 0.07 | 0.75 | 0.32 | 2.56 | 58              | 0.11 | 0.01 | 0.39 | 0.04 | 0.00 | 0.30 | 1.49 | 0.51 | 3.45 |
| Myeloperoxidase Status      |                      |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| Negative                    | 13                   | 0.05 | 0.02 | 0.09 | 0.15 | 0.03 | 0.67 | 3.82 | 0.63 | 21.90 | 12              | 0.10 | 0.01 | 0.24 | 0.03 | 0.00 | 0.65 | 1.36 | 0.75 | 4.93 |
| Positive                    | 58                   | 0.05 | 0.00 | 0.13 | 0.01 | 0.00 | 0.08 | 0.66 | 0.31 | 2.38 | 55              | 0.09 | 0.01 | 0.30 | 0.04 | 0.00 | 0.26 | 1.22 | 0.30 | 3.39 |
| Sample Type                 |                      |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| Pre-chemotherapy Sample     | 32                   | 0.04 | 0.00 | 0.08 | 0.04 | 0.00 | 0.08 | 0.31 | 0.30 | 2.42 | 31              | 0.10 | 0.01 | 0.59 | 0.04 | 0.01 | 0.44 | 1.05 | 0.21 | 3.24 |
| Post-chemotherapy Sample    | 50                   | 0.05 | 0.00 | 0.13 | 0.02 | 0.00 | 0.08 | 0.93 | 0.35 | 5.63 | 46              | 0.10 | 0.01 | 0.26 | 0.04 | 0.00 | 0.27 | 1.73 | 0.70 | 4.04 |
| Remission Status            |                      |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| Resistant                   | 32                   | 0.05 | 0.00 | 0.14 | 0.01 | 0.00 | 0.09 | 0.70 | 0.25 | 2.35 | 31              | 0.12 | 0.01 | 0.30 | 0.05 | 0.00 | 0.33 | 1.48 | 0.44 | 3.98 |
| Relapse                     | 15                   | 0.00 | 0.00 | 0.08 | 0.01 | 0.00 | 0.06 | 0.34 | 0.24 | 0.69 | 15              | 0.03 | 0.00 | 0.53 | 0.03 | 0.00 | 0.29 | 0.99 | 0.18 | 3.40 |
| Persistent Remission        | 35                   | 0.04 | 0.00 | 0.12 | 0.04 | 0.00 | 0.59 | 2.64 | 0.44 | 6.54 | 31              | 0.10 | 0.03 | 0.27 | 0.02 | 0.00 | 0.33 | 1.73 | 0.72 | 4.04 |
| Survival Status             |                      |      |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
| Dead                        | 37                   | 0.01 | 0.00 | 0.08 | 0.01 | 0.00 | 0.07 | 0.48 | 0.26 | 1.60 | 35              | 0.05 | 0.00 | 0.30 | 0.04 | 0.00 | 0.26 | 0.99 | 0.31 | 3.24 |
| Alive                       | 42                   | 0.07 | 0.02 | 0.14 | 0.02 | 0.00 | 0.42 | 2.12 | 0.43 | 5.43 | 39              | 0.12 | 0.03 | 0.59 | 0.03 | 0.00 | 0.28 | 1.53 | 0.70 | 3.90 |

In this study we observed a high marrow LRP expression predicting reduced relapse rate and better 1-year DFS and OS. A diagnosis of APL was another favorable predictor, in agreement with the scientific literature. Although, expression of LRP correlated positively in bone marrow and peripheral blood, the results of blood samples did not correlate with clinical outcome, thus suggesting a possible differential role of tissue-specific gene expression in this regard. Patients with low marrow LRP responded poorly, relapsed and had less survival likelihood than those with high expression. Neither MDR1 nor MRP2 expression in marrow or blood could predict remission, relapse, and 1-year DFS or OS. The strengths of our study include inclusion of a single type of disease and treatment protocol, utilization of both bone marrow and peripheral blood separately without pooling them together, prospective follow up of the patients, and a sample size larger than many other such studies. Being a single-center.
study is a limitation of our study. Please see Supplementary Table 1 for a summary of scientific evidence discussed in this section.

MDR1 and AML Therapeutic Outcome. Several studies have reported MDR1 expression in association with therapeutic outcome in various cancers. In agreement with our findings some studies reported no effect of MDR1 expression on clinical outcome in AML patients treated with different anticancer drugs (n = 30)\(^\text{18}\), or in a non-homogenous group of acute leukemias (AML + ALL), although an inverse relationship with 2-year OS was noted in acute leukemias (n = 71)\(^\text{10}\).

However, some studies with a larger number of AML patients (n = 211, 331) have related MDR1 overexpression with a lower CR rate\(^\text{5,6}\), albeit using a heterogenous patient population, different treatment protocols and less sensitive techniques such as semi-quantitative RT-PCR or flowcytometry. No effect on DFS or OS was observed by one of those studies despite better CR among those who had lower MDR1 expression as well as favorable cytogenetic markers (and vice versa)\(^\text{6}\), while the other study reported no effect of MDR1 expression among the subpopulation (n = 123/331) who were treated like patients in our study\(^\text{5}\). Interestingly, some studies with a sample size lower than ours but on a different drug protocol have shown that MDR1 overexpression correlated with lower CR and higher relapse rates in acute leukemia (AL) (n = 44)\(^\text{7}\) and with reduced DFS in acute lymphoblastic leukemia (ALL) patients treated with ALL-BFM 95 protocol (n = 49)\(^\text{19}\). Thus, a clear association observed in a real clinical situation needs further evidence.

Studies on solid tumors treated with chemotherapy protocols different than those for AML or ALL patients, have also exhibited conflicting results. In an ovarian cancer study (n = 61) MDR1 overexpression was found associated with reduced progression free survival (PFS) and OS but not with chemotherapy response\(^\text{5}\). A study

| Parameters | Coefficient | p-value | N   |
|------------|-------------|---------|-----|
| Persistant Remission | 1.000 | <0.001 | 56  |
| Overall Survival (Weeks) | 0.672 | 0.151 | 53  |
| Disease Free Survival (Weeks) | −0.064 | 0.236 | 56  |
| Final Response | 0.151 | 0.185 | 56  |
| MDR1 express_Marrow (M) | 0.100 | 0.272 | 56  |
| MDR1 express_Blood (B) | 0.068 | 0.016 | 56  |
| MRP2 express_Marrow (M) | 0.037 | 0.393 | 50  |
| MRP2 express_Blood (B) | 0.258 | 0.308 | 46  |
| LRP express_Marrow (M) | 0.157 | 0.302 | 46  |
| LRP express_Blood (B) | 0.112 | 0.077 | 46  |
| p-value | . | <0.001 | . |
| N | 56 | 56 | 56 |

Table 3. Spearman’s Correlation between various variables and gene expression in bone marrow and peripheral blood. Note that ‘M’ denotes Bone Marrow and ‘B’ denotes Peripheral Blood specimen.
| Parameters             | Groups                                                                 | N  | χ² Value | p-value | Odds Ratio | 95% CI Lower | 95% CI Upper |
|-----------------------|------------------------------------------------------------------------|----|----------|---------|------------|--------------|--------------|
| AML Classification    | APL vs. All others                                                     |    |          |         |            |              |              |
| Gender                | Male                                                                   | 61 | 0.159    | 0.690   | 1.286      | 0.372        | 4.446        |
|                       | Female                                                                 | 23 |          |         |            |              |              |
| MPO                   | Negative                                                              | 12 | 13.692   | <0.001  | 11.200     | 2.614        | 47.992       |
|                       | Positive                                                               | 61 |          |         |            |              |              |
| FLT3                  | Negative                                                              | 32 | 0.008    | 1.000   | 1.111      | 0.109        | 11.330       |
|                       | Positive                                                               | 7  |          |         |            |              |              |
| Karyotyping           | Unfavorable                                                           | 17 | 2.378    | 0.165   | 0.281      | 0.053        | 1.503        |
|                       | Favorable                                                             | 28 |          |         |            |              |              |
| Remission Status      | Relapse                                                               | 16 | 6.320    | 0.012   | 0.095      | 0.011        | 0.807        |
|                       | Persistent Remission                                                  | 34 |          |         |            |              |              |
| Survival Status       | Dead                                                                  | 39 | 4.279    | 0.052   | 0.286      | 0.083        | 0.980        |
|                       | Alive                                                                 | 42 |          |         |            |              |              |
| Final Response        | Poor                                                                  | 50 | 15.513   | <0.001  | 0.091      | 0.024        | 0.353        |
|                       | Good                                                                  | 34 |          |         |            |              |              |

**Persistent Remission (Relapse vs. Persistent Remission)**

| Gender                | Male                                                                   | 45 | 0.036    | 1.000   | 0.875      | 0.221        | 3.464        |
|                       | Female                                                                 | 11 |          |         |            |              |              |
| AML Classification    | APL (M3)                                                              | 15 | 6.320    | 0.019   | 0.095      | 0.011        | 0.807        |
|                       | Others                                                                 | 35 |          |         |            |              |              |
| MPO Status            | Negative                                                              | 11 | 5.184    | 0.033   | 0.112      | 0.013        | 0.966        |
|                       | Positive                                                               | 36 |          |         |            |              |              |
| FLT3                  | Negative                                                              | 26 |          |         |            |              |              |
|                       | Positive                                                               |    |          |         |            |              |              |
| Karyotyping           | Unfavorable                                                           | 11 | 1.239    | 0.450   | 0.413      | 0.085        | 2.001        |
|                       | Favorable                                                             | 21 |          |         |            |              |              |
| Survival Status       | Deceased                                                              | 63 | 1.819    | 0.177   | 0.514      | 0.194        | 1.362        |
|                       | Alive                                                                 | 23 |          |         |            |              |              |
| AML Classification    | APL (M3)                                                              | 16 | 4.279    | 0.052   | 0.286      | 0.083        | 0.980        |
|                       | Others                                                                 | 65 |          |         |            |              |              |
| MPO Status            | Negative                                                              | 13 | 1.049    | 0.306   | 0.530      | 0.156        | 1.806        |
|                       | Positive                                                               | 61 |          |         |            |              |              |
| FLT3                  | Negative                                                              | 33 | 1.558    | 0.407   | 0.333      | 0.056        | 1.971        |
|                       | Positive                                                               | 7  |          |         |            |              |              |
| Karyotyping           | Unfavorable                                                           | 17 | 0.061    | 0.805   | 0.860      | 0.260        | 2.843        |
|                       | Favorable                                                             | 30 |          |         |            |              |              |
| Remission Status      | Relapse                                                               | 19 | 23.922   | <0.001  | 28.125     | 6.162        | 128.360      |
|                       | Persistent Remission                                                  | 34 |          |         |            |              |              |
| Final Response        | Poor                                                                  | 52 | 30.929   | <0.001  | 20.357     | 6.071        | 68.262       |
|                       | Good                                                                  | 34 |          |         |            |              |              |

**Final Response (Poor vs. Good)**

| Gender                | Male                                                                   | 66 | 1.929    | 0.165   | 0.494      | 0.181        | 1.350        |
|                       | Female                                                                 | 24 |          |         |            |              |              |
| AML Classification    | APL (M3)                                                              | 17 | 15.513   | <0.001  | 0.091      | 0.024        | 0.353        |
|                       | Others                                                                 | 67 |          |         |            |              |              |
| MPO Status            | Negative                                                              | 14 | 8.050    | 0.007   | 0.177      | 0.049        | 0.635        |
|                       | Positive                                                               | 62 |          |         |            |              |              |
| FLT3                  | Negative                                                              | 35 | 5.169    | 0.033   | invalid    |              |              |
|                       | Positive                                                               | 7  |          |         |            |              |              |
| Karyotyping           | Unfavorable                                                           | 18 | 0.385    | 0.535   | 0.688      | 0.210        | 2.250        |
|                       | Favorable                                                             | 31 |          |         |            |              |              |
| Survival Status       | Dead                                                                  | 42 | 30.929   | <0.001  | 20.357     | 6.071        | 68.262       |
|                       | Alive                                                                 | 44 |          |         |            |              |              |

**Gene Expression:**

| Remission Status      | Relapse vs Persistent Remission                                      |    |          |         |            |              |              |
|                       | Continued                                                            |    |          |         |            |              |              |
on breast cancer (n = 59) reported MDR1 overexpression in patients with decreased response and PFS. Another study on breast cancer patients (n = 220) reported undetectable or very low MDR1 by immunohistochemistry and RT-PCR. Yet another study reported no association of MDR1 overexpression with a clinical outcome in breast cancer tissue (n = 54) compared to normal breast tissue.

One in vitro study has reported changes in MDR1 expression after exposure to cytarabine in both drug-resistant and sensitive leukemic cells, but this could not be related to a change in clinical outcome for obvious reasons. Similarly, another study conducted on breast cancer cell lines as well as breast cancer specimens (n = 168), demonstrated no significant change in MDR1 expression after anthracycline chemotherapy.

In our study we observed that patients with 'AML without maturation' had higher MDR1 expression in marrow compared to 'AML with maturation'. In a previous study on 13 different cell lines it was observed that MDR1 was overexpressed in CD34+ AML cells compared to CD34- cells. Thus, it appears that MDR1 may be associated with a specific subset of AML patients, which partly explains the conflicting results in the scientific literature.

Recently, research has focused on finding an effective MDR1 inhibitor. However without a clear understanding of the role of MDR1, it may not achieve better clinical results.

### MRP2 and AML Therapeutic Outcome

MRP2 is also implicated to drug resistance in hematological as well as solid tumors, although with conflicting results similar to those described above for MDR1. MRP2 overexpression is associated with relapse in AML patients (n = 30) and with lower 2-year survival in acute leukemias.

| Parameters | Groups | N | χ² Value | p-value | Odds Ratio | 95% CI Lower | 95% CI Upper |
|------------|--------|---|----------|---------|------------|--------------|--------------|
| MDR1 expression - Marrow | Low (<1) | 47 | 1.368 | 0.545 | invalid | | |
| | High (≥1) | 3 | | | | | |
| MRP2 expression - Marrow | Low (<1) | 46 | 0.052 | 1.000 | 1.313 | 0.125 | 13.744 |
| | High (≥1) | 4 | | | | | |
| LRP expression - Marrow | Low (<1) | 11 | 1.127 | 0.001 | 10.000 | 2.317 | 43.160 |
| | High (≥1) | 28 | | | | | |
| MDR1 expression - Blood | Low (<1) | 30 | 0.024 | 1.000 | 0.963 | 0.156 | 5.954 |
| | High (≥1) | 6 | | | | | |
| MRP2 expression - Blood | Low (<1) | 10 | 0.406 | 1.000 | 2.074 | 0.211 | 20.367 |
| | High (≥1) | 5 | | | | | |
| LRP expression - Blood | Low (<1) | 6 | 1.328 | 0.249 | 2.078 | 0.593 | 7.275 |
| | High (≥1) | 27 | | | | | |

Table 4. Chi-square analysis and Odds ratios between various variables. All df = 1.
et al. some other studies also could not find any association of MRP2 with chemotherapy outcome, such as in breast renal papillary cell carcinoma (denoted as KIRP), higher LRP or MVP expression is associated with significantly database-generated Kaplan-Meier curves showed that in invasive carcinoma of breast (denoted as BRCA) and survival among high LRP expressors, but only when the first and last quartiles were considered. The Cox coefficients patients (n = 49, n = 27) with reduced RFS in ALL patients (n = 105)\textsuperscript{26}, as well as with poor response to chemotherapy comprising of 5-flurouracil, doxorubicin and cisplatin in esophageal squamous cell carcinoma\textsuperscript{25}. Some \textit{in vitro} studies have demonstrated a correlation between overexpression of MR2P and resistance to antineoplastic drugs\textsuperscript{8,12}. Normally, MR2P expression on hepatocytes is much greater than in other tissues. A study of rat hepatocytes showed that MR2P negative cells showed high sensitivity when treated with cisplatin due to high intracellular platinum accumulation, but when tested in ovarian cancer patients, they did not find this effect\textsuperscript{87}. Similarly, some other studies also could not find any association of MR2P with chemotherapy outcome, such as in breast cancer patients (n = 59) treated with either anthracyclines or hormone therapy or both\textsuperscript{16}, or in ovarian carcinoma patients (n = 61)\textsuperscript{8} treated with different protocols that included platinum-containing drugs. Our results are in agreement with such studies as we found no association between MR2P expression and any therapeutic outcome. Hence it could be possible that MR2P may play a role in drug efflux and thereby in drug resistance in a tissue specific manner, such as liver, but not in AML.

**LRP and AML Therapeutic Outcome.** As described earlier, LRP and vaults play an important role in nucleocytoplasmic transport, apoptosis, DNA damage repair, cellular detoxification and chemotherapy resistance\textsuperscript{8,25}. Some animal and \textit{in vitro} studies reported no association of LRP expression with resistance to cytotoxic drugs\textsuperscript{30,31}. However, Mashima \textit{et al.}\textsuperscript{32} suggested that doxorubicin can bind vRNA which can then be transported by vaults between cytoplasm and nucleus. Another \textit{in vitro} study suggests that LRP transports doxorubicin out of nucleus, resulting in the observed resistance to apoptosis following doxorubicin treatment and is reversed by \textit{in vitro} inhibition of LRP, vPARP and TEP1\textsuperscript{33}. As described earlier, vaults have MVP, vPARP, TEP1 and vRNA as part of their structure. TEP1 forms telomeres and thus prevent cancer formation. Interestingly, we found significant differences in bone marrow but not in peripheral blood samples, which might be suggestive of a role of LRP in combating the carcinogenesis at the initial stage of disease development, especially in hematopoietic stem cells. In fact, it has been postulated that premature aging in normal hematopoietic stem cells induced by chemotherapy or ionizing radiation may result in growth advantage for malignant cells\textsuperscript{34}. The aging is minimized by telomerase activity, and thus increased MVP expression may favor growth of normal bone marrow. However, only clinical studies have the potential to prove its implication in terms of therapeutic response. Some studies reported no association of LRP expression with chemotherapy outcome in AML patients (n = 331, 352)\textsuperscript{6,8} or ALL patients (n = 49, n = 27)\textsuperscript{19,35}. However, patients studied by Schaich \textit{et al.}\textsuperscript{8} received double induction chemotherapy with higher dose of daunorubicin (60 mg/kg/m2/d) as compared to patients in our study (45 mg/kg/m2/d). Such differences in chemotherapy doses could influence the outcome as described by Afsar \textit{et al.}\textsuperscript{36}.

On the other hand, several studies point towards the role of LRP in adverse therapeutic outcomes. Positive LRP expression correlated with lower CR rate but not with relapse rate in acute leukemias\textsuperscript{18}. It also correlated with poor response and prognosis and lower OS in testicular tumor (n = 70)\textsuperscript{17}, and lung cancer (n = 92)\textsuperscript{97}. LRP overexpression is associated with reduced CR rate in AML patients (n = 67)\textsuperscript{68}, decreased DFS in pediatric AML patients (n = 30)\textsuperscript{8}, and poor prognosis in breast cancer patients (n = 59)\textsuperscript{16}. However, results of many such studies should be regarded with caution due to different sample sizes, different analysis methods, or differences in tumor biology or treatment.

Our results disagree with many studies described above. Hence, we explored online OncoLnc\textsuperscript{®} database (http://www.oncolnc.org/search_results/?q = mvp) for further evidence about LRP (MVP). The database-generated Kaplan-Meier curves showed that in invasive carcinoma of breast (denoted as BRCA) and renal papillary cell carcinoma (denoted as KIRP), higher LRP or MVP expression is associated with significantly better survival, thus agreeing with our results. Sarcoma (denoted as SARC) also showed significantly better survival among high LRP expressors, but only when the first and last quartiles were considered. The Cox coefficients for all three diseases (BRCA: −0.23; KIRP: −0.37; SARC: −0.34; all p-values < 0.05) also supported such findings, but their adjusted p-values (q-values) failed to reach statistical significance. The database also shows that in AML

| Parameters | B   | S.E. | Wald | df | p   | Exp(B) | 95% CI Lower | 95% CI Upper |
|------------|-----|-----|------|----|-----|--------|--------------|--------------|
| AML Class (APL/Others) | 2.427 | 1.070 | 5.143 | 1  | 0.023 | 11.328 | 1.390 | 92.303 |
| MPO | 0.578 | 0.921 | 0.394 | 1  | 0.530 | 1.783 | 0.293 | 10.838 |
| Bone Marrow (Gene expression, low vs. high) | | | | | | | | |
| −MR2P | −1.412 | 1.519 | 0.864 | 1  | 0.353 | 0.244 | 0.012 | 4.783 |
| −LRP | −1.843 | 0.771 | 5.708 | 1  | 0.017 | 0.158 | 0.035 | 0.718 |
| Peripheral Blood (Gene expression, low vs. high) | | | | | | | | |
| −MDR1 | −0.152 | 1.167 | 0.017 | 1  | 0.897 | 0.859 | 0.087 | 8.460 |
| −MR2P | −1.276 | 1.324 | 0.930 | 1  | 0.335 | 0.279 | 0.021 | 3.734 |
| −LRP | −0.095 | 0.829 | 0.013 | 1  | 0.908 | 0.909 | 0.179 | 4.619 |
| Constant | 0.743 | 2.114 | 0.123 | 1  | 0.725 | 2.101 | 0.152 | 8.443 |
(denoted as LAML; comprised of a mixed patient population, with a lower sample size) a high LRP expression is associated with poor survival, however statistical significance was not achieved unless at least the top and bottom one third of gene expression values were considered while constructing the survival curve online. The survival curves are given as Supplementary Fig. 2. As LRP is a part of vault structure, the role of LRP as a favorable predictor in AML chemotherapy can be explained on the basis that LRP (and vaults) may be involved in transporting anticancer drugs inside the nucleus. However, further studies are needed to verify this hypothesis.

To conclude, in AML patients treated with standard dose 3+7 cytarabine and daunorubicin regimen, MDR1 and MRP2 gene expression in bone marrow and peripheral blood samples have no association with remission,
resistance or relapse, nor with 1-year DFS or OS. However, higher bone marrow expression of LRP predicts better CR rate, persistent remission and 1-year DFS and OS. Additionally, our model of logistic regression endorses LRP and APL as significant predictors for a good chemotherapeutic response. To the best of our knowledge, our results are the first to show that LRP expression is a predictor of favorable outcome in a commonly used AML chemotherapy.

Further research is warranted to explore the mechanism and regulation of LRP expression, and its interaction with other molecular pathways. Studies are also needed to evaluate the role of LRP as a predictor in different cancers and chemotherapy protocols. We also recommend that further studies with a larger sample size and better techniques should be conducted to clarify the role of xenobiotic transporters in chemotherapy resistance and clinical outcomes.

**Methods**

We recruited 135 AML patients, newly diagnosed according to WHO criteria and treated at National Institute of Blood Diseases and Bone Marrow Transplantation (NIBD&BMT), Karachi, during 2011–2017. All prospective AML patients, including acute promyelocytic leukemia (APL) patients, were included if they received an induction chemotherapy comprising only of the standard 3 + 7 regimen (daunorubicin 45 mg/m² on days 1–3; cytarabine 200 mg/m² on days 1–7). Bone marrow (BM) and blood samples of patients were collected separately. 45 patients were excluded for other reasons, such as hemolyzed samples or no RNA yield. Thus, a total of 90 AML patients were included. Sample collection, storage, enrichment, RNA extraction and reverse transcription reaction were carried out as described previously. The study was approved by the Ethical Review Board at NIBD&BMT in accordance with the Declaration of Helsinki. A written informed consent to participate in this research was given by all patients, or by legal guardians if the patient was below the age of 18-years.

Chemotherapy response, which included complete remission (CR) after first induction chemotherapy, resistance, relapse, overall survival (OS), and disease-free survival (DFS), was defined as described by Döhner et al. 3.

**Real-Time/Quantitative Polymerase Chain Reaction (qPCR).** We used Eco Illumina System version 5.0.16.0 (Illumina, CA, USA). A commercially available VeriQuest Probe qPCR Master Mix (Affymetrix, CA, USA) was used. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression remained internal control in the experiments. Primers and probes were purchased from Integrated DNA Technologies (IDT, IA, USA). The reporter dye in the probe was 6-carboxyfluorescein (FAM) and the quencher was 6-carboxytetramethylrhodamine (Tamra) with an intermediate ZEN-BQI. The primers and probes used for MDR1 were: for – forward 5′-GGAGCCATGTGTTGATTAATTA-3′, reverse 5′-GAAAGCCTTCCTGCGTCTCTTTCTG-3′, probe 5′/-56-FAM/TGAAACTGC/ZEN/CTCAGATGTTTTATGTGTCTACCT/3IABkFQ/-3′; for MRP2 were: forward 5′-ATGCTTCTGGTGGATTATGAT-3′, reverse 5′-TCAAGGCGACGGATAACT-3′, probe 5′/-56-FAM/TGTATCTGT/ZEN/TCAGATGTTTTATGTGTCTACCT/3IABkFQ/-3′; for LRP were: forward 5′-CAGCTGGCCATCGAGATCA-3′, reverse 5′-TCCAGTCTCTGACGGCTAC-3′, reverse 5′/-56-FAM/CAACTCCCA/ZEN/GGAAGCGGCGGC/3IABkFQ/-3′, and for GAPDH were: forward 5′-GAAAGTGAAGTGGACGATTCA-3′, reverse 5′-GAAGATGGTGATGGATTTCC-3′, reverse 5′/-56-JOEN/CCGACTCTT/ZEN/GCCCTTCGAAC/3IABkFQ/(TAMRA)-3′. The reaction conditions and details were described previously.

**Statistical Analysis.** Data was analyzed using SPSS ver. 19.0 software. Qualitative variables were given as frequency and percentage while quantitative variables were described using medians and interquartile ranges where appropriate. Gene expression was calculated from assay Cq values normalized to healthy control blood samples using 2-ΔΔCt. As the gene expression data was not normally distributed, patients with gene expression < 1 were categorized as low expressers, while those with gene expression > 1 were categorized as high expressers. For non-parametric variables, Chi-square test of independence or Fisher Exact test was carried out, and odds ratios were computed where appropriate. Spearman’s correlation was computed between gene expression and clinical outcome. Binary logistic regression analysis was carried out to estimate the predictive value of our model. Kaplan-Meier analysis (log-rank test) was used to estimate 1-year OS and DFS. Only a p-value < 0.05 was considered significant.

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Author Contributions
B.K.: Conception and design, development of methodology, acquisition, analysis, interpretation of data and writing the manuscript. T.S.S.: Review of the manuscript, administrative, technical, and material support and study supervision N.A.A.: Analysis and interpretation of data, writing and review of the manuscript.

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