

## ONLINE SUPPLEMENTARY DATA

*Supplementary Table 1.* Genotype frequencies and minor allele frequencies (MAF). Please note, that for some analyses the polymorphic allele may be more common than the wild type. Genotyping failure rate for *SLC19A1* rs1051266 and *MTRR* rs1801394 was 5 % and less than 2.5 % for all other SNP. One sample had 3 and two samples had 2 unsuccessful analyses, all other samples had the maximum of 1 unsuccessful genotype analysis.

| Gene                     | SNP                        | Genotype                      | N              | MAF (%) |
|--------------------------|----------------------------|-------------------------------|----------------|---------|
| <b>Folate pathway</b>    |                            |                               |                |         |
| <i>MTHFR</i>             | rs1801133<br>(p.Ala262Val) | CC<br>CT<br>TT                | 48<br>55<br>13 | 34.9    |
|                          | rs1801131<br>(p.Glu429Ala) | AA<br>AC<br>CC                | 52<br>56<br>9  | 31.6    |
| <i>MTR</i>               | rs1805087<br>(p.Asp868Gly) | AA<br>AG<br>GG                | 83<br>32<br>4  | 16.8    |
| <i>MTRR</i>              | rs1801394<br>(p.Ile22Met)  | AA<br>AG<br>GG                | 15<br>55<br>42 | 62.1    |
| <i>MTHFD1</i>            | rs2236225<br>(p.Arg709Gln) | GG<br>GA<br>AA                | 37<br>55<br>23 | 43.9    |
| <i>TYMS</i>              | rs34743033                 | 2R + 2R<br>2R + 3R<br>3R + 3R | 28<br>45<br>43 | 56.5    |
| <b>Adenosine pathway</b> |                            |                               |                |         |
| <i>ATIC</i>              | rs2372536<br>(p.Thr116Ser) | CC<br>CG<br>GG                | 47<br>61<br>10 | 34.3    |
| <i>AMPD1</i>             | rs17602729<br>(p.Gln45Ter) | CC<br>CT<br>TT                | 88<br>25<br>3  | 13.4    |
| <i>ITPA</i>              | rs1127354<br>(p.Pro32Thr)  | CC<br>CA<br>AA                | 107<br>12<br>0 | 5.0     |
| <b>MTX transporters</b>  |                            |                               |                |         |
| <i>ABCG2</i>             | rs2231142<br>(p.Q141K)     | CC<br>CA<br>AA                | 92<br>27<br>0  | 11.3    |
|                          | rs2231137<br>(p.V12M)      | GG<br>GA<br>AA                | 111<br>8<br>0  | 3.4     |
| <i>ABCC2</i>             | rs717620<br>(c.-24C>T)     | GG<br>GA<br>AA                | 71<br>41<br>7  | 23.1    |
|                          | rs2804402<br>(c.-1019A>G)  | CC<br>CT<br>TT                | 22<br>68<br>28 | 52.5    |

|                |                                |    |    |  |      |
|----------------|--------------------------------|----|----|--|------|
|                | rs2273697<br>(p.Val417Ile)     | GG | 72 |  | 19.9 |
|                |                                | GA | 45 |  |      |
|                |                                | AA | 1  |  |      |
| <i>ABCB1</i>   | rs1045642<br>(p.Ile1145)       | CC | 33 |  | 48.3 |
|                |                                | CT | 57 |  |      |
|                |                                | TT | 29 |  |      |
| <i>SLC19A1</i> | rs1051266<br>(p.His27Arg)      | AA | 21 |  | 56.3 |
|                |                                | AG | 56 |  |      |
|                |                                | GG | 35 |  |      |
| <i>SLCO1B1</i> | rs11045879<br>(c.1865+4846T>C) | TT | 74 |  | 20.6 |
|                |                                | TC | 41 |  |      |
|                |                                | CC | 4  |  |      |
|                | rs4149056<br>(p.Val174Ala)     | TT | 77 |  | 19.7 |
|                |                                | TC | 37 |  |      |
|                |                                | CC | 5  |  |      |
|                | rs2306283<br>(p.Asn130Asp)     | AA | 28 |  | 48.7 |
|                |                                | AG | 66 |  |      |
|                |                                | GG | 25 |  |      |

*Supplementary Table 2.* Number of samples with at least one polymorphic allele for all performed analyses.

| SNP                        | Number of successful analyses | Response to MTX:<br>Responders / Non - responders | Patients not switched to biological therapy / Patients switched to biological therapy | Patients that did not reach inactive disease / Patients that reached inactive disease | Patients that did not reach remission on therapy / Patients that reached remission on therapy | Patients that did not experience GI AE / Patients that experienced GI AE | Patients that did not experience liver associated AE / Patients that experienced liver associated AE |
|----------------------------|-------------------------------|---|---|---|---|--|--|
| <i>RFC1</i><br>rs1051266   | 117                           | 48 / 32   | 43 / 39   | 22 / 60   | 44 / 38   | 55 / 27  | 60 / 22  |
| <i>MTHFR</i><br>rs1801133  | 116                           | 42 / 24   | 29 / 39   | 20 / 48   | 37 / 31   | 47 / 21  | 51 / 17  |
| <i>MTHFR</i><br>rs1801131  | 117                           | 41 / 23   | 40 / 25   | 18 / 47   | 33 / 32   | 43 / 22  | 48 / 17  |
| <i>MTR</i><br>rs1805087    | 119                           | 23 / 13   | 23 / 13   | 10 / 26   | 22 / 14   | 25 / 11  | 31 / 5   |
| <i>MTRR</i><br>rs1801394   | 112                           | 56 / 58   | 50 / 47   | 30 / 67   | 55 / 42   | 64 / 33  | 71 / 26  |
| <i>MTHFD1</i><br>rs2236225 | 115                           | 42 / 35   | 39 / 39   | 24 / 54   | 43 / 35   | 59 / 19  | 61 / 17  |
| <i>TYMS</i><br>rs34743033  | 116                           | 53 / 32   | 46 / 42   | 28 / 60   | 49 / 39   | 57 / 31  | 66 / 22  |
| <i>ATIC</i><br>rs2372536   | 118                           | 41 / 28   | 39 / 32   | 22 / 49   | 36 / 35   | 47 / 24  | 53 / 18  |
| <i>AMPD1</i><br>rs17602729 | 116                           | 15 / 12   | 16 / 12   | 7 / 23  | 14 / 14   | 13 / 15  | 23 / 5   |
| <i>ITPA</i><br>rs1127354   | 119                           | 8 / 4   | 7 / 5   | 2 / 10  | 6 / 6   | 9 / 3  | 9 / 3  |
| <i>ABCG2</i><br>rs2231142  | 119                           | 14 / 13   | 11 / 16   | 9 / 18  | 13 / 14   | 20 / 7   | 19 / 8   |
| <i>ABCG2</i>               | 119                           | 4 / 4   | 3 / 5   | 3 / 5   | 4 / 4   | 6 / 2  | 7 / 1  |

|                              |     |         |         |         |         |         |         |
|------------------------------|-----|---------|---------|---------|---------|---------|---------|
| rs2231137                    |     |         |         |         |         |         |         |
| <i>ABCC2</i><br>rs717620     | 119 | 25 / 22 | 24 / 24 | 15 / 33 | 28 / 20 | 31 / 17 | 38 / 10 |
| <i>ABCC2</i><br>rs2804402    | 117 | 59 / 34 | 51 / 45 | 29 / 67 | 55 / 41 | 66 / 30 | 73 / 23 |
| <i>ABCC2</i><br>rs2273697    | 118 | 22 / 23 | 19 / 27 | 18 / 28 | 25 / 21 | 32 / 14 | 34 / 12 |
| <i>ABCB1</i><br>rs1045642    | 119 | 48 / 35 | 42 / 44 | 26 / 60 | 49 / 37 | 56 / 30 | 63 / 23 |
| <i>SLCO1B1</i><br>rs11045879 | 119 | 27 / 18 | 23 / 22 | 15 / 30 | 25 / 20 | 26 / 19 | 35 / 10 |
| <i>SLCO1B1</i><br>rs4149056  | 119 | 27 / 15 | 21 / 21 | 14 / 28 | 24 / 18 | 25 / 17 | 34 / 8  |
| <i>SLCO1B1</i><br>rs2306283  | 119 | 53 / 35 | 46 / 45 | 27 / 64 | 48 / 43 | 60 / 31 | 67 / 24 |

*Supplementary Table 3.* Pharmacogenetic determinants of MTX treatment efficacy.

| SNP                        | Non-response to MTX |         |          | Time to biological therapy* |         |          | Time to inactive disease |         |          | Time to remission on therapy |         |          |
|----------------------------|---------------------|---------|----------|-----------------------------|---------|----------|--------------------------|---------|----------|------------------------------|---------|----------|
|                            | Univariate          |         | Cox reg. | Univariate                  |         | Cox reg. | Univariate               |         | Cox reg. | Univariate                   |         | Cox reg. |
|                            | OR; 95 % CI         | p value | OR       | HR; 95 % CI                 | p value | HR       | HR; 95 % CI              | p value | HR       | HR                           | p value | HR       |
| <b>Folate pathway</b>      |                     |         |          |                             |         |          |                          |         |          |                              |         |          |
| <i>RFC1</i><br>rs1051266   | 0.91; 0.38-2.30     | 0.836   | 1        | 1.0; 0.56-1.8               | 0.989   | 1        | 1.25; 0.76 – 2.04        | 0.376   | 1        | 0.74                         | 0.333   | 1        |
| <i>MTHFR</i><br>rs1801133  | 0.64; 0.27 - 1.49   | 0.299   | 1        | 1.78; 1-3.15                | 0.050   | 1.41     | 1; 0.64 – 1.56           | 0.999   | 1        | 0.95                         | 0.868   | 1        |
| <i>rs1801131</i>           | 0.66; 0.28 - 1.52   | 0.325   | 1        | 0.58; 0.34 – 0.98           | 0.043   | 1        | 1.26; 0.8 – 1.97         | 0.317   | 1.19     | 1.25                         | 0.432   | 1        |
| <i>MTR</i> rs1805087       | 0.96; 0.38 - 2.28   | 0.921   | 1        | 0.88; 0.47 – 1.65           | 0.693   | 1        | 1.64; 1.02 – 2.63        | 0.042   | 1.58     | 1.52                         | 0.194   | 1        |
| <i>MTRR</i><br>rs1801394   | 1.22; 0.37 - 5.08   | 0.752   | 1        | 1.64; 0.65 – 4.13           | 0.296   | 1.17     | 0.81; 0.43 – 1.51        | 0.509   | 1        | 0.74                         | 0.410   | 1        |
| <i>MTHFD1</i><br>rs2236225 | 1.31; 0.54-3.40     | 0.556   | 1        | 1.0; 0.58-1.82              | 0.935   | 1        | 0.96; 0.6-1.55           | 0.872   | 1        | 0.82                         | 0.519   | 1        |
| <i>TYMS</i><br>rs34743033  | 0.60; 0.25-1.56     | 0.288   | 1        | 1.1; 0.61-2.22              | 0.645   | 1        | 0.88; 0.53-1.44          | 0.604   | 1        | 0.80                         | 0.478   | 1        |
| <b>Adenosine pathway</b>   |                     |         |          |                             |         |          |                          |         |          |                              |         |          |
| <i>ATIC</i><br>rs2372536   | 0.99; 0.43 - 2.33   | 0.984   | 1        | 0.85; 0.5 – 1.46            | 0.562   | 1        | 1.18; 0.76 – 1.85        | 0.457   | 1.14     | 1.63                         | 0.095   | 1        |
| <i>AMPD1</i><br>rs17602729 | 1.56; 0.61-3.89     | 0.347   | 1        | 0.86; 0.45-1.63             | 0.637   | 1        | 0.99; 0.6-1.64           | 0.974   | 1        | 1.36                         | 0.319   | 1        |

|                           |                   |       |   |                   |       |      |                   |       |         |      |       |   |
|---------------------------|-------------------|-------|---|-------------------|-------|------|-------------------|-------|---------|------|-------|---|
| <i>ITPA</i><br>rs1127354  | 1.57; 0.42 - 5.17 | 0.480 | 1 | 0.92; 0.37 – 2.33 | 0.866 | 1    | 1.6; 0.82 – 3.11  | 0.170 | 1.36    | 0.93 | 0.867 | 1 |
| <b>MTX transporters</b>   |                   |       |   |                   |       |      |                   |       |         |      |       |   |
| <i>ABCG2</i><br>rs2231142 | 1.31; 0.49-3.28   | 0.578 | 1 | 1.26; 0.71-2.26   | 0.429 | 1    | 1.15; 0.68-1.95   | 0.605 | 1       | 1.67 | 0.095 | 1 |
| rs2231137                 | 1.09; 0.19-4.55   | 0.915 | 1 | 1.37; 0.55-3.44   | 0.504 | 1    | 1.0; 0.4-2.47     | 0.993 | 1       | 1.76 | 0.282 | 1 |
| <i>ABCC2</i><br>rs717620  | 1.40; 0.61-3.22   | 0.423 | 1 | 1.13; 0.66-1.92   | 0.665 | 1    | 1.04; 0.67-1.63   | 0.850 | 1       | 0.75 | 0.316 | 1 |
| rs2804402                 | 0.37; 0.14 - 0.99 | 0.048 | 1 | 1.07; 0.55 – 2.08 | 0.84  | 1    | 1.14; 0.65 – 2.01 | 0.640 | 1.07    | 1.25 | 0.508 | 1 |
| rs2273697                 | 1.06; 0.45 - 2.44 | 0.894 | 1 | 1.59; 0.94 – 2.68 | 0.086 | 1.05 | 0.62; 0.39 – 0.99 | 0.043 | 1.55 wt | 0.87 | 0.627 | 1 |
| <i>SLCO1B1</i>            |                   |       |   |                   |       |      |                   |       |         |      |       |   |
| rs11045879                | 0.74; 0.31-1.73   | 0.494 | 1 | 1.07; 0.63-1.84   | 0.799 | 1    | 1.03; 0.65-1.63   | 0.891 | 1       | 1.04 | 0.883 | 1 |
| rs4149056                 | 0.71; 0.28-1.66   | 0.431 | 1 | 1.15; 0.67-1.98   | 0.616 | 1    | 1.1; 0.69-1.75    | 0.682 | 1       | 1.13 | 0.671 | 1 |
| rs2306283                 | 0.82; 0.33-2.16   | 0.671 | 1 | 0.89; 0.46-1.7    | 0.716 | 1    | 0.93; 0.55-1.55   | 0.767 | 1       | 0.79 | 0.494 | 1 |

Legend: \* Patients that were escalated to biologic drugs because of inefficacy or toxicity; Cox reg. – penalized Cox regression; wt – wild type was associated with tested variable; in all other analyses polymorphic allele was associated with tested variable.

*Supplementary Table 4.* Pharmacogenetic determinants of MTX toxicity.

|                            | Time to 1 <sup>st</sup> GI AE |         |                          | Time to 1 <sup>st</sup> liver associated AE |         |                          | MTX discontinuation due to AE*                                  |   |
|----------------------------|-------------------------------|---------|--------------------------|---|---------|--------------------------|---|---|
|                            | Univariate                    |         | Penalized Cox regression | Univariate                                  |         | Penalized Cox regression | Percentage of patients carrying at least one polymorphic allele |   |
|                            | HR; 95 % CI                   | p value | HR                       | HR; 95 % CI                                 | p value | HR                       | Patients, that did not discontinue MTX because of AEs (n=110)   | Patients that discontinued MTX because of AEs (n=7) |
| <b>Folate pathway</b>      |                               |         |                          |   |         |                          |   |   |
| <i>RFC1</i><br>rs1051266   | 0.95; 0.48 – 1.87             | 0.877   | 1                        | 2.09; 0.71 – 6.15                           | 0.180   | 1.09                     | 72 %  | 43 %  |
| <i>MTHFR</i><br>rs1801133  | 1.06; 0.56 – 2.02             | 0.861   | 1                        | 1.74; 0.71 – 4.23                           | 0.224   | 1.12                     | 61 %  | 29 %  |
| rs1801131                  | 1.06; 0.56 – 2.02             | 0.849   | 1                        | 1.11; 0.50 – 2.47                           | 0.801   | 1                        | 53 %  | 100 %   |
| <i>MTR</i><br>rs1805087    | 1.26; 0.63 – 2.50             | 0.515   | 1                        | 0.75; 0.28 – 2.03                           | 0.572   | 1.25 wt                  | 32 %  | 0 %   |
| <i>MTRR</i><br>rs1801394   | 3.16; 0.76 – 13.18            | 0.115   | 1                        | 4.29; 0.58 – 31.85                          | 0.154   | 2.76                     | 86 %  | 100 %   |
| <i>MTHFD1</i><br>rs2236225 | 0.44; 0.22 – 0.85             | 0.015   | 1.55 wt                  | 0.49; 0.21 – 1.16                           | 0.105   | 1.64 wt                  | 68 %  | 71 %  |
| <i>TYMS</i><br>rs34743033  | 1.52; 0.67 – 3.46             | 0.317   | 1                        | 0.95; 0.35 – 2.61                           | 0.925   | 1.01                     | 77 %  | 57 %  |
| <b>Adenosine pathway</b>   |                               |         |                          |   |         |                          |   |   |
| <i>ATIC</i><br>rs2372536   | 1.36; 0.70 – 2.67             | 0.365   | 1                        | 1.42; 0.61 – 3.33                           | 0.414   | 1.24                     | 59 %  | 71 %  |
| <i>AMPD1</i><br>rs17602729 | 2.35; 1.22 – 4.53             | 0.011   | 1.61                     | 0.78; 0.29 – 2.11                           | 0.63    | 1                        | 25 %  | 14 %  |

|                                       |                   |       |   |                   |       |         |  |        |      |
|---------------------------------------|-------------------|-------|---|-------------------|-------|---------|--|--------|------|
| <i>ITPA</i><br>rs1127354              | 0.71; 0.22-2.32   | 0.573 | 1 | 1.31; 0.39-4.43   | 0.668 | 1       |  | 11 %   | 0 %  |
| MTX transporters                      |                   |       |   |                   |       |         |  |        |      |
| <i>ABCG2</i><br>rs 2231142            | 0.64; 0.28 – 1.45 | 0.283 | 1 | 1.22; 0.5 – 2.96  | 0.656 | 1       |  | 23 %   | 14 % |
|                                       | 0.67; 0.16 – 2.76 | 0.575 | 1 | 0.51; 0.07 – 3.80 | 0.509 | 1.54 wt |  | 71.4 % | 0 %  |
| <i>ABCC2</i><br>rs717620<br>rs2804402 | 1.14; 0.6 – 2.15  | 0.694 | 1 | 0.76; 0.33 – 1.74 | 0.515 | 1.29 wt |  | 41 %   | 29 % |
|                                       | 1.06; 0.46 – 2.42 | 0.895 | 1 | 1.58; 0.53 – 4.69 | 0.409 | 1.05    |  | 82 %   | 86 % |
|                                       | 0.99; 0.52 – 1.90 | 0.981 | 1 | 1.03; 0.46 – 2.30 | 0.94  | 1       |  | 41 %   | 0 %  |
| <i>ABCB1</i><br>rs1045642             | 1.87; 0.86 – 4.08 | 0.115 | 1 | 1.53; 0.57 – 4.10 | 0.400 | 1.28    |  | 72 %   | 71 % |
| <i>SLCO1B1</i><br>rs11045879          | 1.93; 1.01 – 3.66 | 0.045 | 1 | 1.34; 0.59 – 3.02 | 0.483 | 1       |  | 37 %   | 57 % |
|                                       | 1.71; 0.89-3.28   | 0.105 | 1 | 1.02; 0.43-2.39   | 0.965 | 1       |  | 34 %   | 57 % |
|                                       | 1.22; 0.54-2.78   | 0.636 | 1 | 1.86; 0.55-6.31   | 0.318 | 1       |  | 76 %   | 86 % |

Legend: wt – wild type was associated with tested variable; in all other analyses polymorphic allele was associated with tested variable \* due to small number of patients (n=7) statistical analyses were unreliable.