MTHFR: methotrexate 5599/5600

677CC = homozygous for MTHFR gene variant with normal activity, 677CT = heterozygous for MTHFR gene variant with reduced activity, 677TT = homozygous for MTHFR gene variant with reduced activity, 1298AA = homozygous for MTHFR gene variant with normal activity, 1298AC = heterozygous for MTHFR gene variant with slightly reduced activity, 1298CC = homozygous for MTHFR gene variant with slightly reduced activity, ALL = acute lymphoblastic leukaemia, CI = confidence interval, cytostat = cytostatic drug, imm sup = immunosuppressant, MTHFR = methylenetetrahydrofolate reductase, NS = non-significant, OR = odds ratio, RR = relative risk (risk ratio), S = significant

Brief summary and justification of choices:
Both methotrexate and MTHFR affect folic acid metabolism. Reduced activity of the enzyme MTHFR results in decreased intracellular tetrahydrofolate concentrations, whilst the toxicity of methotrexate can be reduced by administration of the tetrahydrofolate precursors folic acid or leucovorin (folinic acid). For these reasons, gene variants that result in reduced MTHFR enzyme activity could influence the effectiveness and toxicity of methotrexate.
Gene variant 677C>T
All 6 meta-analyses investigating the effectiveness of methotrexate found no effect of the 677C>T gene variant (Yao 2019 (relapse: 806 patients, survival 513 patients), Shao 2017 (2373 patients), Qiu 2017 Sci Rep (1256 patients), Chen 2017 (2255 patients), Morgan 2014 (812 patients), and Owen 2013 (1375 patients)).
The 13 meta-analysis investigating adverse events (7 in rheumatoid arthritis patients, 5 in cancer patients and 1 in patients with different indications for methotrexate therapy (rheumatoid arthritis, cancer and haematopoietic stem cell transplantation)) did not show a consistent effect. Four did not show an effect of 677C>T gene variant, including one large meta-analysis in rheumatoid arthritis patients (Qiu 20017 Medicine, 3271 patients receiving methotrexate monotherapy). Of the other 9 meta-analyses, some showed only an effect in certain subgroups, and also concerning these subgroups there was little consistency between meta-analyses. In addition, there was not always full consistency between different genetic comparisons within an article. There are indications for stronger effect of the 677C>T gene variant on adverse events in case of folate supplementation, and for a stronger effect on serious adverse events than on mild adverse events. At the moment, however, the evidence for these subgroups is also insufficient.
For these reasons, the KNMP Pharmacogenetics Working Group decided that there is insufficient evidence for a gene-drug interaction (no/no-interactions).
Detailed description of the effects on toxicity that were found
For *rheumatoid arthritis*, the largest meta-analysis (Shao 2017: 25 studies with a total of 4063 patients for 677CT+677TT and 18 studies for 677CT and 677TT) found an overall increase of adverse events for 677CT+677TT and 677TT, but not for 677CT. Adverse events were also increased in one of the three largest subgroups, studies with 100% folate supplementation (10 and 8 studies), for 677CT+677TT, 677CT, and 677TT. For 677CT+677TT, but not for 677CT and 677TT, the risk was also increased for the subgroup of methotrexate monotherapy (5 and 3 studies). For 677TT, but not for 677CT+677TT and 677CT, the risk was also increased in another one of the three largest subgroups: Whites (14 and 11 studies). No significant effect was present in the subgroup of studies with partial folate supplementation (8 and 5 studies). There were no or not enough studies without folate supplementation to perform a meta-analysis. ORs and P-values were numerically lower for 100% folate supplementation than for all studies, suggesting a genuine effect of folate supplementation and not just an effect of power of the subgroups, although differences were small for 677CT+677TT (OR = 1.57 versus 1.36 and P-value = 0.030 versus 0.033). Shao 2017 did not investigate whether the differences between 100% folate supplementation and partial folate supplementation or all studies were significant, so whether folate supplementation increases the effect of the 677C>T gene variant on methotrexate adverse events. Data from two other meta-analyses partially support an increased effect with folate supplementation. Song 2014 found an increased effect for 677TT (3 studies with 100% folate supplementation), but not for 677CT+677TT (4 studies with 100% folate supplementation). The article of Spyridopoulou 2012 investigating multiple methotrexate indications (2-32 studies per meta-analysis with a mean number of 116 patients per study) found an increased risk for adverse events for 677TT and 677CT in studies with 100% folate supplementation (6 studies) and not in studies without or with partial folate supplementation (11 and 3 studies respectively). However, no increased risk of adverse events was found for 677CT+677TT in studies with 100% folate supplementation (7

studies). In Shao 2017, no significant effect was present in the subgroup of studies with mixed therapy (8 and 5 studies). However, a larger effect in methotrexate monotherapy was contradicted by the fourth largest meta-analysis (Qiu 2017 Medicine: 20 studies with a total of 3271 patients on methotrexate monotherapy). This meta-analysis did not find an increase in adverse events for 677CT+677TT, neither in all patients, nor in different ethnicities. In Shao 2017, no significant effect was present in the subgroups of studies with ethnicities other than Whites (7 and 4 studies for East-Asians, 1 study for the other ethnicities, with data only being available for 677CT+677TT in South-Asians). None of the other meta-analyses found a significant effect for Whites (Huang 2020, Qiu 2017 Medicine, Chen 2017, Song 2014, and Owen 2013).
The second largest meta-analysis (Huang 2020: 23 studies with a total of 3817 patients for 677CT+677TT, 18 studies for 677TT and 19 studies for the 677T-allele) found no overall increase of adverse events for 677CT+677TT, 677TT, and the 677T-allele. For 677TT, an increase was only found when compared to 677CC+677CT instead of 677CC, which should theoretically result in a smaller and thus less robust difference. No significant effects were found in 9 European studies, but an increased risk was found for 677CT+677TT in 7 East-Asian studies with a total of 1143 patients (an increased risk for 677TT was only found compared to 677CC+677CT (6 studies)). This effect in East-Asians was contradicted by Shao 2017, Qiu 2017 Medicine, and Song 2014, It was supported for non-Whites by the meta-analysis of Chen 2019.
The third largest meta-analysis (Chen 2017: 20 studies with a total of 3458 patients for 677CT+677TT, 16 studies with a total of 2694 patients for the 677T-allele, and 15 studies with a total of 2660 patients for 677TT compared to 677CC+677CT) found an overall increase of adverse events for 677CT+677TT, the 677T-allele, and for 677TT compared to 677CC+677CT. There was no effect of the gene variant on adverse events in Whites (13 studies with a total of 2133 patients). For non-Whites an increase in adverse events was found for 677CT+677TT (7 studies with a total of 1325 patients) and for 677TT compared to 677CC+677CT (4 studies with a total of 917 patients), but not for the 677T-allele compared to the 677C-allele (5 studies with a total of 951 patients). There was no significant effect of the gene variant on separate adverse events.
The fourth largest meta-analysis (Qiu 2017 Medicine: 20 studies with a total of 3271 patients on methotrexate monotherapy) did not find an increase in adverse events for 677CT+677TT, neither in all patients, nor in different ethnicities.
The fifth largest meta-analysis (Song 2014: 11 studies with a total of 2085 patients for 677CT+677TT, and 7 studies with a total of 1393 patients for 677TT) found an overall increase of adverse events for 677TT, but not for 677CT+677TT. For 677TT an increased risk was also observed for the subgroup of studies with 100% folate supplementation (3 studies) (and further only in two subgroups for which only 1 study was available (Europeans, and discontinuation of methotrexate as outcome)). For 677CT+677TT, a significant effect was only found in the subgroup of studies with discontinuation of methotrexate as outcome (only 2 studies), not in the subgroups of studies with 100% folate supplementation (4 studies) and Europeans (3 studies). A significant effect was not found in East-Asians (4 studies for 677TT and 5 for 677CT+677TT).
The one but smallest and smallest meta-analyses (Owen 2013: 13 studies with a total of 2043 patients, and Fisher 2009: 8 studies with a total of 1441 patients) did not find an increase in adverse events for 677CT+677TT.
Finally, the article of Spyridopoulou 2012, investigating multiple methotrexate indications, (2-32 studies per meta-analysis with a mean number of 116 patients per study) did not find an effect of the 677C>T gene variant on adverse events in rheumatoid arthritis patients (13 studies for 677CT+677TT, and 6 studies for both 677TT and 677CT).
For *cancer*, the largest meta-analysis (Hagleitner 2014: 7 studies with a total of 1044 patients) did not find an increase of hepatotoxicity grade 3-5 for 677CT+677TT. An increase was only found in the subgroup of female patients.
The second largest meta-analysis (Zhu 2018: 2-7 studies with a total of 46-918 patients per meta-analysis) found an increase of hepatotoxicity grade 3-5 for 677CT+677TT (7 studies with a total of 918 patients), but not for 677TT (6 studies with a total of 453 patients). There was also an increase of haematological toxicity grade 3-4 for 677CT+677TT (7 studies with a total of 855 patients), but significance was lost after exclusion of one study not mentioning the methotrexate dose. In addition, there was no increase for 677TT (3 studies with a total of 189 patients). Mucositis grade ≥ 3 was increased for both 677CT+677TT (2 studies with a total of 75 patients) and 677TT (2 studies with a total of 46 patients), There was no increase in hepatotoxicity grade 1-4 (2 studies with 55 patients for 677CT+677TT, and no studies for 677TT), haematological toxicity grade 1-4 (5 studies with 385 patients for 677CT+677TT, and 4 studies with 105 patients for 677TT), and mucositis grade 1-4 (3 studies with 345 patients for 677CT+677TT, and 2 studies with 102 patients for 677TT).
The third largest meta-analysis (Yao 2019: 3-9 studies with a total of 179-820 patients per meta-analysis) did not find found an increase of overall adverse events (4 studies, 820 patients), hepatotoxicity (9 studies, 602 patients), neutropenia (6 studies, 630 patients), mucositis (6 studies, 447 patients), and gastrointestinal adverse events (3 studies, 179 patients) for 677CT+677TT. For neutropenia and hepatotoxicity, an increase was also not found for the subgroups of studies in children and studies with a high methotrexate dose. For hepatotoxicity, there was an increase in the subgroup of studies in acute lymphoblastic lymphoma (5 studies, 446 patients).
The fourth largest meta-analysis (Lopez-Lopez 2013: 2-6 studies with a total of 192-757 patients per meta-analysis) did not find found an increase of hepatotoxicity (6 studies, 757 patients), mucositis (4 studies, 484 patients),

thrombocytopenia (3 studies, 381 patients), neutropenia, anaemia, and leukopenia (2 studies each, 192-221 patients) for 677TT compared to 677CC+677CT.
The smallest meta-analysis (Yang 2012: 2-7 studies per meta-analysis with a mean of 71 patients per study) found an increase in hepatotoxicity (6 studies) for 677CT+677TT, but not for 677TT and 677CT. Oral mucositis (6 studies) and myelosuppression (3 studies) were increased for 677TT, but not for 677CT. Gastrointestinal toxicity (4 studies) was increased for 677TT. There was no increase in haematological toxicity (3 studies), in skin toxicity, neurotoxicity, and neutropenia (2 studies each).
Finally, the article of Spyridopoulou 2012, investigating multiple methotrexate indications, (2-32 studies per meta-analysis with a mean number of 116 patients per study) did not find an effect of the 677C>T gene variant on adverse events in patients with haematological conditions (9 studies for 677CT+677TT, and 7 studies for both 677TT and 677CT).
For *mixed indications*, the article of Spyridopoulou 2012 (2-32 studies per meta-analysis with a mean number of 116 patients per study) found an increase in hepatotoxicity for 677CT+677TT and 677TT, but not for 677CT (9 studies for 677CT+677TT, and 5 studies for both 677TT and 677CT). There was an increase in adverse events in studies with 100% folate supplementation for 677TT and for 677CT, but not for 677CT+677TT (7 studies for 677CT+677TT, and 6 studies for both 677TT and 677CT).There was an decrease in graft-versus-host disease for 677TT, but not for 677CT and 677CT+677TT (7 studies for 677CT+677TT, and 2 studies for both 677TT and 677CT). The same was true for overall adverse events in haematopoietic stem cell transplantation (5 studies for 677CT+677TT, and 3 studies for both 677TT and 677CT). There was an increase in neurotoxicity for 677TT compared to 677CC+677CT, but not for 677CT+677TT for neurotoxicity (4 studies for 677CT+677TT, and 2 studies for both 677TT compared to 677CC+677CT). There was no effect of the 677C>T gene variant on the occurrence of more than 1 type of adverse events (32 studies for 677CT+677TT, and 20 studies for both 677TT and 677CT), the occurrence of more than 2 types of adverse events (14 studies and 8 studies), mucositis (5 and 4 studies) haematological toxicity (3 studies for 677TT and 677CT), and gastrointestinal toxicity.
<u>Gene variant 1298A>C</u>
The 1298A>C gene variant results in an enzyme in which the activity is less severely reduced than for the 677C>T gene variant. In accordance with this, there is not much evidence for an effect for this gene variant.
The meta-analyses of Huang 2020, Yao 2019, Zhu 2018, Qiu 2017 Medicine, Chen 2017, Owen 2013, and Fisher 2009 did not find an effect of 1298A>C on adverse events. The meta-analysis of Fan 2019 found a decreased risk for adverse events for 1298CC based on 3 studies in which not all patients received folate, but an increased risk in 1298AC and 1298AC+1298CC based on 2 studies with mixed ethnicity. Song 2014 found a decreased risk of discontinuation of methotrexate for 1298AC+1298CC, but this was only based on 1 study. Lopez-Lopez 2013 found a decreased leukopenia risk for 1298CC, but this was only based on 2 studies and no effect of the 677C>T gene variant on leukopenia risk was observed. Yang 2012 found a decreased risk of skin toxicity for 1298AC+1298CC, but this was only based on 2 studies and was not observed for 677CT+677TT. Spyridopoulou 2012 found an increased risk of toxicity for 1298CC based on four studies in which no patients received folate, but no effect of the 677C>T variant in studies without folate supplementation. The small number of studies, the opposite directions of the effects in Fan 2019, and the lack of confirmation by the 677C>T gene variant suggest these results to be chance findings.
The meta-analyses of Morgan 2013, and Owen 2013 did not find an effect of 1298A>C on response. The meta-analysis of Fan 2019 found an increased response for 1298AC in East-Asians, but this was only based on 1 study. The meta-analyses of Qiu 2017 Sci Rep and Chen 2017 found a decreased response for 1298CC compared to 1298AA+1298AC in respectively South-Asians and non-Whites, both based on 2 studies and both not confirmed by an effect for 677TT compared to 677CC+677CT. The small number of studies and the lack of confirmation by the 677C>T gene variant suggest these results to be chance findings.
In addition to the evidence for an effect of 1298A>C on the outcome of methotrexate therapy being insufficient, there are indications for a linkage disequilibrium between 1298A>C and 677C>T. This means that the effect of 1298A>C may not be independent of the effect of 677C>T. For these reasons, there is no cause for inclusion of this gene variant in the MTHFR pharmacogenetic interactions.

Source	Code	Effect	Comments
ref. 1 - imm sup Huang J et al. Are gene polymorphisms related to adverse events of methotrexate in patients with	3	Meta-analyses of the effect of 677C>T and 1298A>C on adverse events of methotrexate in rheumatoid arthritis patients. 23 studies with a total of 3817 patients investigated 677C>T. All 23 studies were included in the meta-analysis comparing 677CT+677TT with 677CC. 18 studies were included in the meta-analyses comparing 677TT with 677CC, and comparing 677TT with 677CC+677CT. 19 studies were included in the meta-analysis comparing the 677T-allele with the 677C-allele. Of the 23 studies, 9 were in Europeans and 7 in East-Asians. The total number of patients in the East-Asian studies	Authors' conclusion: ''Evidence-based results suggest that the MTHFR 677C>T (rs1801133), ATIC 347C>G (rs2372536),

<table>
<tr>
<td>rheumatoid arthritis? A retrospective cohort study based on an updated meta-analysis.
Ther Adv Chronic Dis 2020;11:20406 22320916026.
PMID: 32426102.

ref. 1, continuation</td>
<td></td>
<td>was 1143.
20 studies investigated 1298A>C. All 20 studies were included in the meta-analysis comparing 1298AC+1298CC with 1298AA. 19 studies were included in the meta-analyses comparing 1298CC with 1298AA, comparing 1298CC with 1298AA+1298AC, and comparing the 1298C-allele with the 1298A-allele. Of the 20 studies, 8 were in Europeans, 5 in East-Asians and 2 in Africans.
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Quality of the included studies was not judged.
Publication bias analysis was not performed.

Results:
<table>
<tr><td colspan="3">Adverse events compared to 677CC:</td></tr>
<tr><td></td><td>677CT+677TT</td><td>677TT</td></tr>
<tr><td rowspan="3">all patients</td><td rowspan="2">NS</td><td>trend for an increase (p = 0.067) (NS)</td></tr>
<tr><td>Compared to 677CC+ 677CT, the increase was significant: OR = 1.44 (95% CI: 1.14-1.81) (S).</td></tr>
<tr><td colspan="2">The result for the 677T-allele compared to the 677C-allele was NS.</td></tr>
<tr><td rowspan="3">Europeans</td><td rowspan="2">NS</td><td>NS</td></tr>
<tr><td>Compared to 677CC+ 677CT, the result was also NS.</td></tr>
<tr><td colspan="2">The result for the 677T-allele compared to the 677C-allele was NS.</td></tr>
<tr><td rowspan="3">East-Asians</td><td rowspan="2">OR = 2.01 (95% CI: 1.002-4.032) (S)</td><td>trend for an increase (p = 0.073) (NS)</td></tr>
<tr><td>Compared to 677CC+ 677CT, the increase was significant: OR = 1.70 (95% CI: 1.18-2.45) (S).</td></tr>
<tr><td colspan="2">For the 677T-allele compared to the 677C-allele, there was a trend for an increase (p = 0.078) (NS).</td></tr>
<tr><td colspan="3">Heterogeneity between studies was absent for 677TT compared to 677CC+677CT.
There was significant heterogeneity between studies for the other 3 comparisons (677CT+677TT compared to 677CC, 677TT compared to 677CC, and the 677T-allele compared to the 677C-allele).</td></tr>
</table>

<table>
<tr><td colspan="3">Adverse events compared to 1298AA:</td></tr>
<tr><td></td><td>1298AC+1298CC</td><td>1298CC</td></tr>
<tr><td rowspan="3">all patients</td><td rowspan="2">NS</td><td>NS</td></tr>
<tr><td>Compared to 1298AA+ 1298AC, the result was also NS.</td></tr>
<tr><td colspan="2">The result for the 1298C-allele compared to the 1298A-allele was NS.</td></tr>
<tr><td rowspan="2">Europeans</td><td rowspan="2">NS</td><td>NS</td></tr>
<tr><td>Compared to 1298AA+ 1298AC, the result was also NS.</td></tr>
</table>
</td>
<td>677TT: C

677CT +TT: C

1298A C+CC: AA

1298C C: AA</td>
<td>RFC-1 80G>A (rs1051266), ABCB1 3435C>T (rs1045642) polymorphisms are associated with methotrexate-related toxicity.''</td>
</tr>
</table>

<table>
<tr><td>ref. 1, continuation</td><td></td><td>
<table>
<tr><td></td><td colspan="2">The result for the 1298C-allele compared to the 1298A-allele was NS.</td></tr>
<tr><td rowspan="3">East-Asians</td><td rowspan="2">NS</td><td>NS</td></tr>
<tr><td>Compared to 1298AA+ 1298AC, the result was also NS.</td></tr>
<tr><td colspan="2">The result for the 1298C-allele compared to the 1298A-allele was NS.</td></tr>
<tr><td rowspan="3">Africans</td><td rowspan="2">NS</td><td>NS</td></tr>
<tr><td>Compared to 1298AA+ 1298AC, the result was also NS.</td></tr>
<tr><td colspan="2">The result for the 1298C-allele compared to the 1298A-allele was NS.</td></tr>
<tr><td colspan="3">Heterogeneity between studies was absent for:
- East-Asians (all four comparisons)
- 1298CC compared to 1298AA+1298AC in Europeans and Africans
- 1298CC compared to 1298AA in Europeans
Heterogeneity between studies was low for:
- 1298CC compared to 1298AA in Africans
- 1298CC compared to 1298AA+1298AC in all patients
Heterogeneity was moderate to high for the other comparisons.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 2 - cytostat
Yao P et al. The influence of MTHFR genetic polymorphisms on adverse reactions after methotrexate in patients with hematological malignancies: a meta-analysis. Hematology 2019;24:10-9. PMID: 30024839.</td><td>3</td><td>Meta-analyses of the effect of 677C>T and 1298A>C on adverse events and effectiveness of methotrexate in patients with haematological malignancies. Only good quality studies (scoring more than 6 points on the 9-point Newcastle-Ottawa Quality Assessment Scale) were included in the meta-analyses.17 studies with a total of 2133 patients were included. All 17 studies provided data on 677C>T and adverse events and 13 studies provided data on 1298A>C and adverse events.
For 677C>T, the meta-analysis investigating all adverse events included 4 studies with a total of 820 patients, the meta-analysis investigating neutropenia included 6 studies, of which 5 in children and 4 with high-dose methotrexate, with a total of 630 patients, the meta-analysis investigating hepatotoxicity included 9 studies, of which 6 in children, 5 with high-dose methotrexate, and 5 in acute lymphoblastic leukaemia (ALL) patients, with a total of 602 patients, the meta-analysis investigating gastrointestinal reaction included 3 studies with a total of 179 patients, the meta-analysis investigating mucositis included 6 studies with a total of 447 patients, the meta-analysis investigating relapse included 5 studies, of which 3 in children, with a total of 806 patients, and the meta-analysis investigating survival included 6 studies, of which 4 in children, with a total of 513 patients.
For 1298A>C, the meta-analysis investigating neutropenia included 5 studies with a total of 481 patients, the meta-analysis investigating hepatotoxicity included 7 studies with a total of 552 patients, the meta-analysis investigating mucositis included 6 studies with a total of 448 patients, the meta-analysis investigating relapse included 4 studies with a total of 323 patients, and the meta-analysis investigating survival included 6 studies, of which 4 in children, with a total of 502 patients.
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Publication bias analysis was not performed.</td><td>Authors' conclusion:
''The polymorphism of MTHFR C677T/A1298C may not be an important indicator for the accurate detection of side effects of chemotherapy after using methotrexate.''</td></tr>
</table>

<table>
<tr>
<td>ref. 2, continuation</td>
<td>677CT +TT: C

1298A C+CC: AA</td>
<td>Results:
<table>
<tr><td colspan="4">Results for 677CT+677TT compared to 677CC:</td></tr>
<tr><td colspan="4">Adverse events</td></tr>
<tr><td colspan="2"></td><td></td><td>incidence for 677CC</td></tr>
<tr><td colspan="2">all adverse events</td><td>NS</td><td></td></tr>
<tr><td rowspan="3">neutropenia</td><td>all</td><td>trend for an increase (p = 0.06) (NS)</td><td>42%</td></tr>
<tr><td>children</td><td>NS</td><td></td></tr>
<tr><td>high-dose</td><td>NS</td><td></td></tr>
<tr><td rowspan="4">hepatotoxicity</td><td>all</td><td>NS</td><td>24%</td></tr>
<tr><td>children</td><td>NS</td><td>22%</td></tr>
<tr><td>high-dose</td><td>NS</td><td></td></tr>
<tr><td>ALL</td><td>RR = 1.92 (95% CI: 1.01-3.67) (S)</td><td></td></tr>
<tr><td colspan="2">gastrointestinal adverse event</td><td>trend for an increase (p = 0.06) (NS)</td><td></td></tr>
<tr><td colspan="2">mucositis</td><td>NS</td><td>26%</td></tr>
<tr><td colspan="4">Effectiveness</td></tr>
<tr><td rowspan="2">relapse</td><td>all</td><td>NS</td><td></td></tr>
<tr><td>children</td><td>NS</td><td></td></tr>
<tr><td rowspan="2">survival</td><td>all</td><td>NS</td><td>78%</td></tr>
<tr><td>children</td><td>NS</td><td></td></tr>
<tr><td colspan="4">Heterogeneity between studies was absent for overall adverse events.
Heterogeneity between studies was low for mucositis.
Heterogeneity between studies was moderate to high for neutropenia, hepatotoxicity, gastrointestinal reaction, relapse and survival.</td></tr>
</table>

<table>
<tr><td colspan="4">Results for 1298AC+1298CC compared to 1298AA:</td></tr>
<tr><td colspan="4">Adverse events</td></tr>
<tr><td colspan="2"></td><td></td><td>incidence for 1298AA</td></tr>
<tr><td colspan="2">neutropenia</td><td>NS</td><td></td></tr>
<tr><td colspan="2">hepatotoxicity</td><td>NS</td><td>36%</td></tr>
<tr><td colspan="2">mucositis</td><td>NS</td><td>22%</td></tr>
<tr><td colspan="4">Effectiveness</td></tr>
<tr><td colspan="2">relapse</td><td>trend for an decrease (p = 0.08) (NS)</td><td></td></tr>
<tr><td rowspan="2">survival</td><td>all</td><td>NS</td><td>83%</td></tr>
<tr><td>children</td><td>NS</td><td></td></tr>
<tr><td colspan="4">Heterogeneity between studies was absent for survival in children.
Heterogeneity between studies was low for neutropenia, hepatotoxicity, mucositis, and relapse.
Heterogeneity between all studies on survival was high.</td></tr>
</table>
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<td></td>
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<tr>
<td>ref. 3 – cytostat
Zhu C et al. Associations between the C677T and A1298C polymorphisms of MTHFR and the toxicity of methotrexate in</td>
<td>3</td>
<td>Meta-analyses of 14 cohort studies investigating the effect of 677C>T and 1298A>C on methotrexate toxicity in children with malignancies. The quality of the included studies ranged from 7-9 points on the 9-point Newcastle-Ottawa Quality Assessment Scale. Methotrexate doses ranged from 1-12 g/m^2.
For 677C>T, the meta-analysis investigating hepatotoxicity grade ≥ 2 contained 7 studies with a total of 918 patients for 677CT+677TT compared to 677CC, 6 studies with a total of 453 patients for 677TT compared to 677CC and 6 studies with a total of 768 patients for 677TT compared to 677CC+677CT, the meta-analysis investigating mucositis grade ≥ 3 contained 2 studies with a total of 75 patients for</td>
<td>Authors' conclusion:
"We found significant associations of the MTHFR C677T polymorphism with hepatotoxicity (grade ≥2), hematological toxicity (grade 3-</td>
</tr>
</table>

<table>
<tr>
<td>childhood malignancies: a meta-analysis. Pharmacogenomics J 2018;18:450-9. PMID: 28696419.

ref. 3, continuation</td>
<td></td>
<td>677CT+677TT compared to 677CC and for 677TT compared to 677CC+677CT, and 2 studies with a total of 46 patients for 677TT compared to 677CC, and the meta-analysis investigating haematological toxicity grade 3-4 contained 7 studies with a total of 855 patients for 677CT+677TT compared to 677CC, 3 studies with a total of 189 patients for 677TT compared to 677CC, and 3 studies with a total of 351 patients for 677TT compared to 677CC+677CT. Of the 6 studies with a total of 768 patients investigating hepatotoxicity grade ≥ 2 in 677TT, 4 with a total of 245 patients (of whom 117 677CT) were in Asians, 1 with a total of 483 patients (of whom 187 677CT) was in a mixed population, and 1 with a total of 40 patients (of whom 11 677CT) was in Africans.
For 1298A>C, the meta-analysis investigating hepatotoxicity grade ≥ 2 contained 4 studies with a total of 381 patients for 1298AC+1298CC compared to 1298AA and for 1298CC compared to 1298AA+1298AC, and 4 studies with a total of 240 patients for 1298CC compared to 1298AA, the meta-analysis investigating mucositis grade ≥ 3 contained 2 studies with a total of 77 patients for 1298AC+1298CC compared to 1298AA and for 1298CC compared to 1298AA+1298AC, and 2 studies with a total of 48 patients for 1298CC compared to 1298AA, and the meta-analysis investigating haematological toxicity grade 3-4 contained 3 studies with a total of 289 patients for 1298AC+1298CC compared to 1298AA, 2 studies with a total of 194 patients for 1298CC compared to 1298AA, and 3 studies with a total of 218 patients for 1298CC compared to 1298AA.
Of the 14 studies in this meta-analysis, 11 were also included in the meta-analysis of Yao 2019.
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Publication bias analysis was not performed.

Results:
<table>
<tr><td colspan="4">Results compared to 677CC:</td></tr>
<tr><td></td><td>677CT+677TT</td><td>677TT</td><td>incidence for 677CC</td></tr>
<tr><td>hepatotoxicity (grade ≥ 2)</td><td>RR = 1.22 (95% CI: 1.01-1.49) (S)</td><td>NS
Results were also NS in Asians (4 studies) and in the study with a mixed population. However, results were S in the small African study: RR = 11.1 (95% CI: 1.72-100) (S).
Compared to 677CC+677CT, the result was also NS for all patients and in the study with a mixed population, but S for Asian patients (4 studies)</td><td>27%</td></tr>
</table>
</td>
<td>4) in a dominant genetic model and mucositis (grade ≥ 3) in all models. No significant association was found with the MTHFR A1298C polymorphism. For children with malignancy, genotyping of the MTHFR C677T polymorphism is expected to be a useful tool in reducing toxicity and improving outcome in personalized methotrexate therapy.''</td>
</tr>
</table>

ref. 3, continuation				(RR = 1.39 (95% CI: 1.01-1.92) (S)) and in the small African study (RR = 20.0 (95% CI: 3.03-100) (S)).		
		hepatotoxicity (grade 1-4)	NS	-		
				Compared to 677CC+677CT, the result was NS.		
	677CT +TT: D 677TT: D	mucositis (grade ≥ 3)	RR = 5.56 (95% CI: 1.15-25) (S)	RR = 10 (95% CI: 2.0-50) (S)	4.5%	
				Compared to 677CC+677CT, the result was also S: RR = 10 (95% CI: 3.1-33) (S)		
		mucositis (grade 1-4)	NS	NS		
				Compared to 677CC+677CT, the result was also NS.		
		haematologic toxicity (grade 3-4)	RR = 1.54 (95% CI: 1.03-2.27) (S)	NS	30%	
			Heterogeneity between studies was diminished, but significance of the RR was lost after excluding one study not showing the dose (NS).	Of the 3 included studies (all in acute lymphoblastic leukaemia patients), the one in Africans found an increased risk (RR = 4.55 (95% CI: 1.19-16.7) (S)), the one in Caucasians a decreased risk (RR = 0.35 (95% CI: 0.13-0.95) (S)), and the one in Asians no significant effect (NS).		
				Compared to 677CC+677CT, the result was also NS. Of the 3 included studies (all in acute lymphoblastic leukaemia patients), the one in Africans found an increased risk (RR = 3.33 (95% CI: 1.41-7.69) (S)), the one in Caucasians a decreased risk (RR = 0.37 (95% CI: 0.14-0.96) (S)), and the one in Asians no significant effect (NS).		
		haematologic toxicity (grade 1-4)	NS	NS		
				Compared to 677CC+677CT, the result was also NS.		

<table>
<tr>
<td>ref. 3, continu-ation</td>
<td>1298AC+CC: AA

1298CC: AA</td>
<td>
Heterogeneity between studies was absent for:
- hepatotoxicity grade ≥ 2 for 677CT+677TT compared to 677CC in all patients, for 677TT compared to 677CC in Asians, and for 677TT compared to 677CC+677CT in Asians
- hepatotoxicity grade 1-4 for both comparisons
- mucositis grade ≥ 3 for all three comparisons
- mucositis grade 1-4 for all three comparisons
- haematologic toxicity grade 1-4 for all three comparisons
Heterogeneity between studies was moderate to high for:
- hepatotoxicity grade ≥ 2 for 677TT compared to 677CC in all patients, and for 677TT compared to 677CC+677CT in all patients
- haematologic toxicity (grade 3-4) for all three comparisons
<table>
<tr><td colspan="3">Results compared to 1298AA:</td></tr>
<tr><td></td><td>1298AC+1298CC</td><td>1298CC</td></tr>
<tr><td>hepatotoxicity (grade ≥ 2)</td><td>NS</td><td>NS
Compared to 1298AA+1298AC, the result was also NS.</td></tr>
<tr><td>mucositis (grade ≥ 3)</td><td>NS</td><td>NS
Compared to 1298AA+1298AC, the result was also NS.</td></tr>
<tr><td>mucositis (grade 1-4)</td><td>NS
Heterogeneity between studies became low, but the RR remained NS after excluding one study without Hardy-Weinberg equilibrium (NS).</td><td>NS
Heterogeneity between studies disappeared, but the RR remained NS after excluding one study with a small sample size (NS).
Compared to 1298AA+1298AC, the result was also NS.
Heterogeneity between studies disappeared, but the RR remained NS after excluding one study without Hardy-Weinberg equilibrium (NS).</td></tr>
<tr><td>haematologic toxicity (grade 3-4)</td><td>NS</td><td>NS
Compared to 1298AA+1298AC, the result was also NS.</td></tr>
<tr><td>haematologic toxicity (grade 1-4)</td><td>NS</td><td>NS
Compared to 1298AA+1298AC, the result was also NS.</td></tr>
<tr><td colspan="3">Heterogeneity between studies was absent for:
- hepatotoxicity grade ≥ 2 for all three comparisons
- mucositis grade ≥ 3 for all three comparisons
- haematologic toxicity grade 3-4 for 1298AC+1298CC compared to 1298AA
- haematologic toxicity grade 1-4 for 1298AC+1298CC compared to 1298AA
Heterogeneity between studies was moderate to high for:
- mucositis grade 1-4 for all three comparisons
- haematologic toxicity grade 3-4 for 1298CC compared to 1298AA, and for 1298CC compared to 1298AA+1298AC
- haematologic toxicity grade 1-4 for 1298CC compared to 1298AA, and for 1298CC compared to 1298AA+1298AC</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

<table>
<tr>
<td>ref. 4 - imm sup
Fan H et al. Lack of association between MTHFR A1298C polymorphism and outcome of methotrexate treatment in rheumatoid arthritis patients: evidence from a systematic review and meta-analysis. Int J Rheum Dis 2017;20:526-40. PMID: 28544525.

ref. 4, continuation</td>
<td>3

1298CC: AA#

1298A</td>
<td>Meta-analyses of the effect of 1298A>C on toxicity (18 studies with a total of 2777 patients) and effectiveness (10 studies with a total of 1325 patients) of methotrexate in rheumatoid arthritis patients.
For toxicity, the meta-analysis comparing 1298CA+1298CC with 1298AA contained all 18 studies and the meta-analyses for the other comparisons contained 15 studies.
For effectiveness, the meta-analysis comparing 1298CA+1298CC with 1298AA contained all 10 studies and the meta-analyses for the other comparisons contained 8 studies.
Of the 22 studies in this meta-analysis, 14 were also included in the meta-analysis of Huang 2020.
Toxicity was evaluated in the included studies based on all reported methotrexate-related adverse effects or discontinuation of methotrexate due to adverse effects.
Effectiveness was evaluated In the included studies by improvement in the Disease Activity Score of 28 joints based on European League Against Rheumatism criteria (DDAS28/EULAR criteria), methotrexate dose, American College of Rheumatology 20 at 6 months guidelines (ACR20 at 6 months), or other rules designed by authors from different laboratories.
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of no of mild heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Quality of the included studies was not judged.
Potential publication bias was assessed by the Egger’s weighted linear regression test and Begg’s rank correlation test, but only for the comparison of 1298CC with 1298AA+1298AC (15 studies for toxicity and 8 for effectiveness). This comparison was not important enough for this risk analysis to include it in this summary.

Results:
<table>
<tr><td colspan="3">Results compared to 1298AA:</td></tr>
<tr><td colspan="3">Toxicity</td></tr>
<tr><td></td><td>1298AC</td><td>1298CC</td></tr>
<tr><td rowspan="2">all studies</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="3">not 100% folate supplementation</td><td>NS</td><td>OR = 0.38 (95% CI: 0.16-0.94) (S) (3 studies)</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="2">100% folate supplementation</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="3">combined therapy</td><td>trend for a decrease (p = 0.097) (NS)</td><td>NS</td></tr>
<tr><td colspan="2">trend for a decrease (p = 0.089) (NS)</td></tr>
<tr><td rowspan="2">methotrexate monotherapy</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="3">Jewish</td><td>NS</td><td>OR = 0.19 (95% CI: 0.05-0.73) (S) (only 1 study)</td></tr>
<tr><td colspan="2">trend for a decrease (p = 0.057) (NS)</td></tr>
<tr><td rowspan="2">White</td><td>trend for a decrease (p = 0.063) (NS)</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="2">East-Asian</td><td>trend for a decrease (p = 0.064) (NS)</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
</table>
</td>
<td>Authors’ conclusion:
’’Overall, our meta-analysis suggested no significant effect of MTHFR gene A1298C polymorphism on methotrexate outcome in rheumatoid arthritis patients. However, due to several limitations of our meta-analysis, the results should be interpreted cautiously and require further confirmation using high-quality studies.’’</td>
</tr>
</table>

ref. 4, continuation	C: C 1298AC+CC: C	mixed-ethnicity	OR = 2.24 (95% CI: 1.39-3.60) (S) (2 studies)	NS	
			OR = 2.29 (95% CI: 1.46-3.61) (S) (2 studies)		
		Latin American	NS	trend for an increase (p = 0.055) (NS)	
			trend for an increase (p = 0.053) (NS)		
		any adverse effects	NS	NS	
			NS		
		discontinuation of methotrexate	NS	NS	
			NS		
		Effectiveness			
		all studies	NS	NS	
			NS		
		partial folate supplementation	NS	NS	
			NS		
		100% folate supplementation	NS	NS	
			NS		
		combined therapy	NS	NS	
			NS		
		methotrexate monotherapy	NS	NS	
			NS		
		South-Asian	trend for an increase (p = 0.062) (NS)	NS	
			NS		
		White	NS	NS	
			NS		
		East-Asian	OR = 3.37 (95% CI: 1.14-9.99) (S) (only 1 study)	NS	
			NS		
		mixed-ethnicity	NS	NS	
			NS		
		ACR20 at 6 months	NS	NS	
			NS		
		DDAS28/EULAR criteria	NS	NS	
			NS		
		Heterogeneity between studies was moderate to high for: - toxicity in all studies for all three comparisons - toxicity outcome discontinuation of methotrexate for all three comparisons - toxicity in mixed ethnicity for 1298AC compared to 1298AA - toxicity in methotrexate monotherapy for 1298AC+1298CC compared to 1298AA - effectiveness outcome ACR20 at 6 months for 1298AC compared to 1298AA, and for 1298AC+1298CC compared to 1298AA - effectiveness with 100% folate supplementation for 1298CC compared to 1298AA - effectiveness of methotrexate monotherapy for 1298CC compared to 1298AA - effectiveness outcome DDAS28/EULAR criteria for 1298CC compared to 1298AA - effectiveness of combined therapy for 1298AC+1298CC compared to 1298AA - effectiveness in East-Asians for 1298AC+1298CC compared to 1298AA There was only one study in the meta-analysis for: - toxicity in methotrexate monotherapy for 1298AC compared to		

<table>
<tr><td>ref. 4, continuation</td><td></td><td>
<table>
<tr><td>1298AA, and for 1298CC compared to 1298AA
- toxicity in Jews for all three comparisons
- toxicity in Latin Americans for all three comparisons
- effectiveness in East-Asians for 1298AC compared to 1298AA, and for 1298CC compared to 1298AA
- effectiveness in mixed ethnicity for all three comparisons
Heterogeneity between the studies was absent or mild for all other comparisons.</td></tr>
<tr><td>There was no evidence for publication bias for toxicity and effectiveness for 1298CC compared to 1298AA+1298AC (respectively including 15 of the 18 studies and 8 of the 10 studies in the meta-analyses).</td></tr>
<tr><td>For all three genetic comparisons, sensitivity analyses showed pooled ORs to be not significantly affected by the sequential omission of one individual study at a time.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 5 - imm sup
Shao W et al. Association between MTHFR C677T polymorphism and methotrexate treatment outcome in rheumatoid arthritis patients: a systematic review and meta-analysis. Genet Test Mol Biomarkers 2017;21:275-85.
PMID: 28277784.

ref. 5, continuation</td><td>3

677TT: C

677CT: C</td><td>
Meta-analyses of the effect of 677C>T on toxicity and effectiveness of methotrexate in rheumatoid arthritis patients. 25 included studies with a total of 4063 patients investigated toxicity, of which 18 studies reported data for 677CT and 677TT separately. 16 included studies with a total of 2373 patients investigated effectiveness, of which 12 studies reported data for 677CT and 677TT separately.
Of the 32 studies in this meta-analysis, 19 were also included in the meta-analysis of Huang 2020, and 21 in the meta-analysis of Fan 2017.
Meta-analyses were performed with a random-effects model in case of significant heterogeneity between the studies and with a fixed-effects model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent, but the data exaction method was not mentioned.
Quality of the included studies was not judged.
Potential publication bias was assessed with the Egger’s test and Begg’s test, but only for all studies, not for the subgroups.

Results:
<table>
<tr><td colspan="4">Results compared to 677CC:</td></tr>
<tr><td></td><td>677CT</td><td>677TT</td><td>incidence for 677CC</td></tr>
<tr><td colspan="4">Toxicity</td></tr>
<tr><td rowspan="2">all studies</td><td>NS</td><td>OR = 1.61 (95% CI: 1.03-2.50) (S)</td><td rowspan="2">55%</td></tr>
<tr><td colspan="2">OR = 1.36 (95% CI: 1.02-1.80) (S)</td></tr>
<tr><td rowspan="2">partial folate supplementation</td><td>NS</td><td>NS</td><td></td></tr>
<tr><td colspan="2">NS</td><td></td></tr>
<tr><td rowspan="2">100% folate supplementation</td><td>OR = 1.66 (95% CI: 1.01-2.71) (S) (8 studies)</td><td>OR = 2.54 (95% CI: 1.36-4.75) (S) (8 studies)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">OR = 1.57 (95% CI: 1.05-2.36) (S) (10 studies)</td></tr>
<tr><td rowspan="2">mixed therapy method</td><td>NS</td><td>trend for an increase (p = 0.057) (NS)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">trend for an increase (p = 0.079) (NS)</td></tr>
<tr><td rowspan="2">methotrexate monotherapy</td><td>NS</td><td>trend for an increase (p = 0.095) (NS)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">OR = 1.50 (95% CI: 1.01-2.23) (S)</td></tr>
</table>
</td><td>Authors’ conclusion:
’’Our study indicated that the MTHFR C677T polymorphism could be used as a predictor of methotrexate toxicity in rheumatoid arthritis patients. However, large randomized prospective studies will be required to effectively replicate and validate these findings.’’</td></tr>
</table>

ref. 5, continuation			(5 studies)		
		South-Asian	-	-	
			NS		
		Jewish	NS	NS	
			NS		
		White	NS	OR = 1.77 (95% CI: 1.26-2.47) (S) (11 studies)	
			trend for an increase (p =0.058) (NS)		
		East-Asian	NS	NS	
			NS		
		Latin American	NS	NS	
			NS		
		mixed ethnicity	NS	NS	
			NS		
		discontinuation of methotrexate	NS	NS	
			NS		
		Effectiveness			
		all studies	NS	NS	52%
			NS		
		partial folate supplementation	NS	NS	
			NS		
		100% folate supplementation	NS	NS	
			NS		
		mixed therapy method	NS	NS	
			NS		
		methotrexate monotherapy	NS	NS	
			NS		
		South-Asian	NS	NS	
			NS		
		White	NS	NS	
			NS		
		East-Asian	NS	NS	
			NS		
		mixed ethnicity	NS	NS	
			NS		
		DDAS28/EULAR criteria	NS	NS	
			NS		
		ACR20 at 6 months	trend for an increase (p = 0.089) (NS)	NS	
			trend for an increase (p = 0.056) (NS)		
		DAS28 ≤3.2 at 6 months	NS	NS	
			NS		
		Heterogeneity between studies was moderate to high for: - toxicity in all studies for all three comparisons - toxicity with 100% folate supplementation for all three comparisons - toxicity in mixed therapy for all three comparisons - toxicity in East-Asians for all three comparisons - toxicity with partial folate supplementation for 677CT compared to 677CC, and for 677CT+677TT compared to 677CC - toxicity in Whites for 677CT compared to 677CC, and for 677CT+677TT compared to 677CC - effectiveness with partial folate supplementation for 677TT compared to 677CC - effectiveness outcome DAS28 ≤3.2 at 6 months for 677TT compared to 677CC There was only one study in the meta-analysis for: - toxicity in Jews for all three comparisons		

<table>
<tr><td>ref. 5, continuation</td><td></td><td>- toxicity in Latin Americans for all three comparisons
- toxicity in mixed ethnicity for all three comparisons
- toxicity in South-Asians for 677CT+677TT compared to 677CC (no studies for the other comparisons)
- effectiveness in East-Asians for 677CT compared to 677CC, and for 677TT compared to 677CC
- effectiveness in mixed ethnicity for all three comparisons
Heterogeneity between the studies was absent or low for all other comparisons.

There was no evidence for publication bias for toxicity in all three genetic comparisons and for effectiveness for 677CT compared to 677CC, and 677CT+677TT compared to 677CC.
However, for effectiveness for 677TT compared to 677CC, the Egger’s test suggested significant publication bias (p = 0.012). Using the trim and fill method the imputed studies produced a symmetrical funnel plot. The pooled analysis incorporating the hypothetical studies did not significantly alter the association (trend for an decrease (p = 0.08) (NS)).

For all three genetic comparisons, sensitivity analyses showed pooled ORs to be not significantly affected by the sequential omission of one individual study at a time.</td><td></td></tr>
<tr><td>ref. 6 - imm sup
Qiu Q et al. Polymorphisms and pharmacogenomics for the clinical efficacy of methotrexate in patients with rheumatoid arthritis: a systematic review and meta-analysis. Sci Rep 2017;7:44015. PMID: 28266606.</td><td>3

677CT+TT: AA

677TT: AA</td><td>Meta-analyses of the effect of 677C>T and 1298A>C on effectiveness of methotrexate monotherapy in rheumatoid arthritis patients. Only studies published after 2005 were included.
10 included studies with a total of 1256 patients (46% responders) investigated the effect of 677C>T, of which 6 European studies with a total of 861 patients (49% responders), 3 South-Asian studies with a total of 302 patients (31% responders), and 1 East-Asian study with 93 patients (67% responders). 8 included studies with a total of 952 patients (45% responders) investigated the effect of 1298A>C, of which 4 European studies with a total of 558 patients (48% responders), 2 South-Asian studies with a total of 267 patients (28% responders), and 2 East-Asian studies with a total of 127 patients (62% responders).
Of the 10 studies in this meta-analysis, 7 were also included in the meta-analysis of Fan 2017, and 9 in the meta-analysis of Shao 2017.
Meta-analyses were performed with a random-effects model in case of mild to high heterogeneity between the studies and with a fixed-effects model in case of absent or very low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Quality of the included studies was not judged.
Potential publication bias was assessed with the Egger’s test and Begg’s funnel plots, but only one analysis was shown for all 5 comparisons in the article (of which 4 are included in this summary) and it was not mentioned for which comparison the analysis was performed. No publication bias analysis was performed for the subgroups.

Results:
<table>
<tr><td colspan="3">Response compared to 677CC:</td></tr>
<tr><td></td><td>677CT+677TT</td><td>677TT</td></tr>
<tr><td>all patients</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td></td><td>Compared to 677CC+677CT, the result was also NS.</td></tr>
<tr><td></td><td colspan="2">The result for the 677T-allele compared to the 677C-allele was NS.</td></tr>
<tr><td>Europeans</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td></td><td>Compared to 677CC+</td></tr>
</table>
</td><td>Authors’ conclusion:
’’Associations between methotrexate response in rheumatoid arthritis patients in MTHFR 1298A > C (rs1801131), ATIC 347C > G (rs2372536), RFC-1 80G > A (rs1051266), SLC19A1 A > G (rs2838956) and SLC19A1 G > A (rs7499) genetic polymorphisms were found, but not observed between the MTHFR 677C > T (rs1801133), TYMS 28 bp VNTR (rs34743033), MTRR 66A > G (rs1801394), and ABCB1 3435C > T (rs1045642).’’</td></tr>
</table>

ref. 6, continuation				
				677CT, the result was also NS.
			The result for the 677T-allele compared to the 677C-allele was NS.	
		South-Asians	NS	NS
				Compared to 677CC+677CT, the result was also NS.
			The result for the 677T-allele compared to the 677C-allele was NS.	
		East-Asians	NS	NS
				Compared to 677CC+677CT, the result was also NS.
			The result for the 677T-allele compared to the 677C-allele was NS.	
		There was moderate heterogeneity between the studies for the 677T-allele compared to the 677C-allele in Europeans. There was mild heterogeneity between the studies for: - 677TT compared to 677CC in Europeans - 677TT compared to 677CC+677CT in Europeans - the 677T-allele compared to the 677C-allele in all patients Heterogeneity between the studies was absent or very low for the other comparisons.		
		There was no evidence for publication bias.		
	1298AC+CC: AA	Response compared to 1298AA:		
			1298AC+1298CC	1298CC
		all patients	NS	NS
				Compared to 1298AA+1298AC, the result was also NS.
			The result for the 1298C-allele compared to the 1298A-allele was NS.	
		Europeans	NS	NS
				Compared to 1298AA+1298AC, the result was also NS.
			The result for the 1298C-allele compared to the 1298A-allele was NS.	
	1298CC: C	South-Asians (2 studies)	NS	NS
				Compared to 1298AA+1298AC, the result was S: OR = 0.45 (95% CI: 0.23-0.91) (S)
			The result for the 1298C-allele compared to the 1298A-allele was NS.	
		East-Asians	NS	NS
				Compared to 1298AA+1298AC, the result was also NS.
			The result for the 1298C-allele compared to the 1298A-allele was NS.	
		There was moderate to high heterogeneity between the studies for: - 1298CC compared to 1298AA in Europeans - 1298AC+1298CC compared to 1298AA in East-Asians - the 1298C-allele compared to the 1298A-allele in East-Asians There was mild heterogeneity between the studies for: - 1298AC+1298CC compared to 1298AA in all patients		

<table>
<tr><td>ref. 6, continuation</td><td></td><td>- 1298CC compared to 1298AA in South-Asians
- 1298CC compared to 1298AA+1298AC in all patients
- 1298CC compared to 1298AA+1298AC in Europeans
- the 1298C-allele compared to the 1298A-allele in all patients
- the 1298C-allele compared to the 1298A-allele in Europeans
Heterogeneity between studies was absent or low for the other comparisons.
There was no evidence for publication bias.</td><td></td></tr>
<tr><td>ref. 7 - imm sup
Qiu Q et al. Polymorphisms and pharmacogenomics for the toxicity of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic review and meta-analysis.
Medicine (Baltimore) 2017;96:e6337.
PMID: 28296761.</td><td>3

677CT+TT: AA

1298AC+CC: AA</td><td>Meta-analyses of the effect of 677C>T and 1298A>C on toxicity of methotrexate monotherapy in rheumatoid arthritis patients.
20 included studies with a total of 3271 patients (41% with an adverse event) investigated the effect of 677C>T, of which 7 European studies with a total of 1330 patients (33% with an adverse event), 6 East-Asian studies with a total of 1170 patients (49% with an adverse event), 2 South-Asian studies with a total of 184 patients (23% with an adverse event), 1 North-American study, 1 Oceanian study, 1 African study, 1 West-Asian study, and 1 South-American study. 16 included studies with a total of 2447 patients (40% with an adverse event) investigated the effect of 1298A>C, of which 6 European studies with a total of 1140 patients (31% with an adverse event), 4 East-Asian studies with a total of 532 patients (50% with an adverse event), 1 North-American study, 1 Oceanian study, 1 African study, 1 West-Asian study, 1 South-American study, and 1 South-Asian study.
Of the 21 studies in this meta-analysis, 20 were also included in the meta-analyses of Huang 2020 and Shao 2017, and 15 in the meta-analysis of Fan 2017.
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of absent or low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Quality of the included studies was not judged.
Possible publication bias was not investigated.

Results:
Adverse events for 677CT+677TT compared to 677CC:
all patients: NS
Europeans: NS
East-Asians: trend for an increase (p = 0.074) (NS)
South-Asians: NS
There was significant heterogeneity between the studies.

Adverse events for 1298AC+1298CC compared to 1298AA:
all patients: NS
Europeans: NS
East-Asians: NS
There was significant heterogeneity between the studies.</td><td>Authors' conclusion:
"Significant associations were not observed between the MTHFR (677C>T (rs1801133) and 1298A>C (rs1801131)), ATIC 347C>G (rs2372536), MTR 2756A>G (rs1805087), MTRR 66A>G (rs1801394), ABCB1 3435C>T (rs1045642), and RFC-1 80G>A (rs1051266, when all the patients were included) and the toxicity of methotrexate in rheumatoid arthritis patients."</td></tr>
<tr><td>ref. 8 - imm sup
Chen Y et al. Are gene polymorphisms related to treatment outcomes of methotrexate in patients with rheumatoid arthritis? A</td><td>3</td><td>Meta-analyses of the effect of 677C>T and 1298A>C on toxicity and effectiveness of methotrexate in rheumatoid arthritis patients.
For toxicity, 20 included studies with a total of 3458 patients (41% with an adverse event) compared 677CT+677TT with 677CC, of which 13 studies with a total of 2133 patients (42% with an adverse event) in Whites, and 7 studies with a total of 1325 patients (39% with an adverse event) in non-Whites. 16 included studies with a total of 2694 patients (43% with an adverse event) compared the 677T-allele with the 677C-allele, of which 11 studies with a total of 1743 patients (41% with an adverse event) in Whites, and 5 studies with a total of 951 patients (46% with an adverse event) in non-Whites. 15 included</td><td>Authors' conclusion:
"The absence of TYMS 1494 del6 and FPGS rs10106 and presence of MTHFR C677T predict adverse events in rheumatoid arthritis</td></tr>
</table>

systematic review and meta-analysis. Pharmacogenomics 2017;18:175-95. PMID: 27992285. **ref. 8, continuation**		studies with a total of 2660 patients (43% with an adverse event) compared 677TT with 677CC+677CT, of which 11 studies with a total of 1743 patients (41% with an adverse event) in Whites, and 4 studies with a total of 917 patients (46% with an adverse event) in non-Whites. 15 included studies with a total of 2606 patients (38% with an adverse event) compared 1298AC+1298CC with 1298AA, of which 10 studies with a total of 1817 patients (36% with an adverse event) in Whites, and 5 studies with a total of 789 patients (42% with an adverse event) in non-Whites. 13 included studies with a total of 2143 patients (41% with an adverse event) compared the 1298C-allele with the 1298A-allele, of which 9 studies with a total of 1578 patients (38% with an adverse event) in Whites, and 4 studies with a total of 565 patients (50% with an adverse event) in non-Whites. 13 included studies with a total of 2259 patients (43% with an adverse event) compared 1298CC with 1298AA+1298AC, of which 10 studies with a total of 1728 patients (41% with an adverse event) in Whites, and 3 studies with a total of 531 patients (50% with an adverse event) in non-Whites. Meta-analyses of separate adverse events included 2 through 5 studies. For effectiveness, 15 included studies with a total of 2255 patients (51% responders) compared 677CT+677TT with 677CC, of which 10 studies with a total of 1740 patients (50% responders) in Whites, and 5 studies with a total of 515 patients (54% responders) in non-Whites. 12 included studies with a total of 1678 patients (49% responders) compared the 677T-allele with the 677C-allele, of which 9 studies with a total of 1334 patients (52% responders) in Whites, and 3 studies with a total of 344 patients (37% responders) in non-Whites. 11 included studies with a total of 1644 patients (49% responders) compared 677TT with 677CC+677CT, of which 9 studies with a total of 1334 patients (52% responders) in Whites, and 2 studies with a total of 310 patients (36% responders) in non-Whites. 13 included studies with a total of 1826 patients (48% responders) compared 1298AC+1298CC with 1298AA, of which 9 studies with a total of 1258 patients (52% responders) in Whites, and 4 studies with a total of 568 patients (39% responders) in non-Whites. 11 included studies with a total of 1363 patients (47% responders) compared the 1298C-allele with the 1298A-allele, of which 8 studies with a total of 1019 patients (51% responders) in Whites, and 3 studies with a total of 344 patients (37% responders) in non-Whites. 10 included studies with a total of 1329 patients (47% responders) compared 1298CC with 1298AA+1298AC, of which 8 studies with a total of 1019 patients (51% responders) in Whites, and 2 studies with a total of 310 patients (36% responders) in non-Whites. Of the 32 studies in this meta-analysis, 18 were also included in the meta-analyses of Huang 2020, 20 in the meta-analysis of Fan 2017, 29 in the meta-analysis of Shao 2017, 8 in the meta-analysis of Qiu 2017 Sci Rep, and 19 in the meta-analysis of Qiu 2017 Medicine. Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent, but data exaction was not standardised. Dependent on the availability, either genotype frequencies of efficacy and/or toxicity, adjusted ORs, adjusted hazard ratios or unadjusted ORs were extracted. Quality of the included studies was not judged. Possible publication bias was assessed by funnel plot, but only for all studies, not for the subgroups. Results: Results for 677CT+677TT compared to 677CC: *Adverse events*	patients treated with methotrexate. Moreover, variations of the associations were found between Caucasians and non-Caucasians.''

ref. 8, continuation	677CT +TT: C 677TT: C	all studies	OR = 1.41 (95% CI: 1.02-1.94) (S)	
			The result was also S for: - the 677T-allele compared to the 677C-allele: OR = 1.29 (95% CI: 1.02-1.63) (S) - 677TT compared to 677CC+677CT: OR = 1.38 (95% CI: 1.00-1.89) (S)	
		White	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
		Non-White	OR = 1.92 (95% CI: 1.01-3.64) (S) (7 studies)	
			The result was NS for the 677T-allele compared to the 677C-allele.	
			The result was S for 677TT compared to 677CC+677CT: OR = 1.75 (95% CI: 1.13-2.70) (S)	
		gastrointestinal adverse events	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
			The result was also NS in: - Whites (also for the 677T-allele compared to the 677C-allele and for 677TT compared to 677CC+677CT) - non-Whites	
		hepatic adverse events	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
			The result was also NS in: - Whites (also for the 677T-allele compared to the 677C-allele and for 677TT compared to 677CC+677CT) - non-Whites	
		central nervous system adverse events	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
			The result was also NS in non-Whites.	
		dermatologic adverse events	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
			All three studies were in Whites.	
		respiratory adverse events	NS	
			Both studies were in non-Whites.	
		haematologic adverse events	NS	
			All three studies were in non-Whites.	
		discontinuation due to adverse events	NS	
		Response		
		all studies	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
		White	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele	

ref. 8, continuation			- 677TT compared to 677CC+677CT	
		Non-White	NS	
			The result was also NS for: - the 677T-allele compared to the 677C-allele - 677TT compared to 677CC+677CT	
		The heterogeneity between the studies was moderate to high for. - adverse events in all studies, in Whites, and in non-Whites for 677CT+677TT compared to 677CC - adverse events in all studies, in Whites and in non-Whites for the 677T-allele compared to the 677C-allele - gastro-intestinal adverse events in all patients, and in non-Whites for 677CT+677TT compared to 677CC - gastrointestinal adverse events in Whites for 677TT compared to 677CC+677CT - hepatic adverse events in all patients, in Whites, and in non-Whites for 677CT+677TT compared to 677CC - all comparisons for dermatologic adverse events - discontinuation due to adverse events The heterogeneity between the studies was low for. - adverse events in all studies and in non-Whites for 677TT compared to 677CC+677CT - gastrointestinal adverse events in all patients for 677TT compared to 677CC+677CT - hepatic adverse events in Whites for the 677T-allele compared to the 677C-allele The heterogeneity between the studies was absent or very low for. - adverse events in all studies and in non-Whites for 677TT compared to 677CC+677CT - gastrointestinal adverse events in Whites for 677CT+677TT compared to 677CC - gastrointestinal adverse events in all patients and in Whites for the 677T-allele compared to the 677C-allele - hepatic adverse events in all patients for the 677T-allele compared to the 677C-allele - hepatic adverse events in all patients and in Whites for 677TT compared to 677CC+677CT - respiratory adverse events - haematologic adverse events - all comparisons for central nervous system adverse events - all comparisons for response		
		There was no evidence for publication bias for adverse events and for response for 677CT+677TT compared to 677CC.		
	1298AC+CC: AA	Results for 1298AC+1298CC compared to 1298AA:		
		Adverse events		
		all studies	NS	
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC	
		White	NS	
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC	
		Non-White	NS	
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC	
		gastrointestinal adverse events	NS	
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele	

ref. 8, continuation	1298CC: C		- 1298CC compared to 1298AA+1298AC
		hepatic adverse events	NS
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC
		central nervous system adverse events	NS
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC
		Response	
		all studies	NS
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC
		White	NS
			The result was also NS for: - the 1298C-allele compared to the 1298A-allele - 1298CC compared to 1298AA+1298AC
		Non-White	NS
			The result was also NS for the 1298C-allele compared to the 1298A-allele.
			The result was S for 1298CC compared to 1298AA+ 1298AC: OR = 0.42 (95% CI: 0.19-0.94) (S) (2 studies)
		The heterogeneity between the studies was moderate to high for. - adverse events in Whites for 1298AC+1298CC compared to 1298AA - adverse events in all studies and in Whites for the 1298C-allele compared to the 1298A-allele - adverse events in Whites for 1298CC compared to 1298AA+ 1298AC - gastrointestinal adverse events for 1298AC+1298CC compared to 1298AA, and for the 1298C-allele compared to the 1298A-allele - hepatic adverse events for 1298AC+1298CC compared to 1298AA, and for the 1298C-allele compared to the 1298A-allele - response in all studies and in non-Whites for 1298AC+1298CC compared to 1298AA - response in non-Whites for the 1298C-allele compared to the 1298A-allele The heterogeneity between the studies was low for. - adverse events in all studies for 1298AC+1298CC compared to 1298AA - adverse events in non-Whites for the 1298C-allele compared to the 1298A-allele - adverse events in all studies for 1298CC compared to 1298AA+ 1298AC - hepatic adverse events for 1298CC compared to 1298AA+ 1298AC - response in all studies for 1298CC compared to 1298AA+1298AC The heterogeneity between the studies was absent or very low for. - adverse events in non-Whites for 1298AC+1298CC compared to 1298AA - adverse events in non-Whites for 1298CC compared to 1298AA+ 1298AC - gastrointestinal adverse events for 1298CC compared to 1298AA+ 1298AC - central nervous system adverse events - response in Whites for 1298AC+1298CC compared to 1298AA - response in all studies and in Whites for the 1298C-allele compared to the 1298A-allele	

ref. 8, continuation		- response in Whites and in non-Whites for 1298CC compared to 1298AA+1298AC	
ref. 9 - imm sup Song GG et al. Association of the MTHFR C677T and A1298C polymorphisms with methotrexate toxicity in rheumatoid arthritis: a meta-analysis. Clin Rheumatol 2014;33:1715-24. PubMed PMID: 24794492.	4 677TT: C 3 (677CT + 677TT): C 1298CC: AA	Meta-analysis of 12 studies examining the toxicity of methotrexate in a total of 2,288 patients with rheumatoid arthritis. Of the 12 studies in this meta-analysis, 1 was of high quality, 10 of acceptable quality and 1 of low quality according to the Scottish Intercollegiate Guidelines Network (SIGN) guidelines. Of the 12 studies in this meta-analysis, all were also included in the meta-analyses of Huang 2020, Shao 2017 and Qiu 2017 Medicine, 10 in the meta-analysis of Fan 2017, and 11 in the meta-analysis of Chen 2017. Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of absent or low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised. There were no signs of publication bias based on Egger's linear regression test, but this was only shown for two of the six comparisons in the study and it was not mentioned whether this were 677TT compared to 677CC and 1298CC compared to 1298AA or 677TT compared to 677TC+677CC and 1298CC compared to 1298AC+1298AA. No publication bias analysis was performed for the subgroups. 677TT compared to 677CC (7 studies, 1,393 patients): - increase in the risk of toxicity (OR = 1.945; 95% CI: 1.051-3.602) (S). The risk was also increased in the subgroup of patients who received folate(OR = 2.891; 95% CI: 1.850-4.520) (3 studies) and in the only study involving European patients (OR = 2.125; 95% CI: 1.089-4.146) (S). - increase in the risk of discontinuing methotrexate as a result of toxicity (OR = 2.125; 95% CI: 1.089-4.146) (one study) (S) - no difference in the risk of an adverse event (NS) - no difference in the risk of an abnormal liver function test (two studies) (NS) Despite moderate to severe heterogeneity between the studies for all comparisons with more than two studies, all ORs were determined using a fixed effects model. (677CT + 677TT) compared to 677CC (11 studies, 2,085 patients): - increase in the risk of discontinuing methotrexate as a result of toxicity (OR = 1.597; 95% CI: 1.043-2.445) (2 studies) (S) - no difference in the risk of toxicity (NS). There was also no difference in the subgroup of patients who received folate (4 studies) (NS). - no difference in the risk of an adverse event (NS) - no difference in the risk of an abnormal liver function test (NS) For the risk of stopping methotrexate, there was slight heterogeneity between the studies and the OR was determined using the fixed effects model. For the other comparisons, the heterogeneity was moderate to severe and the OR was determined using the random effects model. 1298CC compared to 1298AA (7 studies, approx. 1,200 patients): - no difference in the risk of toxicity (NS) - no difference in the risk of an adverse event (NS) - no difference in the risk of an abnormal liver function test (NS) - no difference in the risk of discontinuing methotrexate as a result of	Authors' conclusion: "The results of our meta-analysis suggest that the MTHFR C677T and A1298C polymorphisms are associated with methotrexate toxicity in rheumatoid arthritis patients."

ref. 9, continuation	3 (1298AC + 1298CC): AA[#]	toxicity (1 study) (NS) For the risk of toxicity and the risk of an adverse event, there was moderate heterogeneity between the studies and the OR was determined using the fixed effects model. For the risk of an abnormal liver function test, there was moderately severe heterogeneity between the two studies and the random effects model was used. (1298AC + 1298CC) compared to 1298AA (8 studies, 1,367 patients): - decrease in the risk of discontinuing methotrexate as a result of toxicity (OR = 0.521; 95% CI: 0.309-0.876) (1 study) (S) - no difference in the risk of toxicity (NS) - no difference in the risk of an adverse event (NS) - no difference in the risk of an abnormal liver function test (NS) The heterogeneity between studies was severe and the ORs were determined using the random effects model.	
ref. 10 - cytostat Hagleitner MM et al. The role of the MTHFR 677C>T polymorphism in methotrexate-induced liver toxicity: a meta-analysis in patients with cancer. Pharmacogenomics J 2014;14:115-9. PubMed PMID: 23648444.	3 (677CT+TT): AA	Meta-analysis of 7 studies examining the hepatotoxicity of methotrexate in a total of 1,044 cancer patients. Only studies in Caucasian population groups were included. Of the 7 studies in this meta-analysis, 2 were also included in the meta-analysis of Yao 2019, and none were included in the meta-analysis of Zhu 2018. Prospective registration of the protocol was not mentioned, but the search and selection strategy was transparent and data exaction was standardised. Quality of the included studies and possible publication bias were not analysed. 677T compared to 677C: - no difference in the risk of grade 3-5 hepatotoxicity (NS). An increase in the risk of hepatotoxicity was found for female patients (OR = 1.71; 95% CI: 1.04-2.81). The heterogeneity between the studies was statistically non-significant and was possibly caused by differences in methotrexate dose and whether or not folate was used.	Authors' conclusion: "In patients with cancer, the MTHFR 677T allele has only a minor role in the development of methotrexate-induced hepatotoxicity."
ref. 11 - imm sup Morgan MD et al. MTHFR functional genetic variation and methotrexate treatment response in rheumatoid arthritis: a meta-analysis. Pharmacogenomics 2014;15:467-75. PubMed PMID: 24624914.	3 677TT: AA 677CT: AA	Meta-analysis of 4 studies examining the effectiveness of methotrexate 10-25 mg/week in a total of 812 patients with rheumatoid arthritis. Only studies in Caucasian population groups were included. Of the 4 studies in this meta-analysis, 2 were also included in the meta-analysis of Fan 2017, 3 in the meta-analyses of Shao 2017 and Chen 2017, and 1 in the meta-analysis of Qiu 2017 Sci Rep. DAS/DAS28 was used as a measure of the effectiveness. DAS28 is the score for disease activity based on 28 joints. DAS28 ≤ 3.2 corresponds to a low disease activity. Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardised. Quality of the included studies and possible publication bias were not analysed. 677TT compared to 677CC: - no difference in the chance of a low disease activity after 6 months of methotrexate (NS) 677CT compared to 677CC: - no difference in the chance of a low disease activity after 6 months of methotrexate (NS) 1298CC compared to 1298AA (3 studies, 692 patients):	Authors' conclusion: "MTHFR SNPs C677T and A1298C are unlikely to have a clinically meaningful effect on the first 6 months of methotrexate treatment in early rheumatoid arthritis."

ref. 11, continuation	1298CC: AA 1298AC: AA	- no difference in the chance of a low disease activity after 6 months of methotrexate (NS) 1298AC compared to 1298AA (3 studies, 692 patients): - no difference in the chance of a low disease activity after 6 months of methotrexate (NS) For 677C>T there was a slight heterogeneity between the studies, for 1298A>C there was moderate heterogeneity.	

ref. 12 - cytostat
Lopez-Lopez E et al.
A systematic review and meta-analysis of MTHFR polymorphisms in methotrexate toxicity prediction in pediatric acute lymphoblastic leukemia.
Pharmacogenomics J 2013;13:498-506.
PubMed PMID: 23089671.
	3
	
	677TT: AA
	
	1298CC: AA[#]
		Meta-analysis of 8 studies examining the hepatotoxicity of methotrexate in a total of 1,042 children with acute lymphatic leukaemia.
		Of the 8 studies included in this meta-analysis, 6 were also included in the meta-analyses by Yao 2019 and Zhu 2018, and none in the meta-analysis by Hagleitner 2014.
		Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardised.
		Quality of the included studies and possible publication bias were not analysed.
		
		677TT compared to (677CC + 677CT):
		- no difference in the risk of:
		  - hepatotoxicity (6 studies, 757 patients) (NS)
		  - mucositis (4 studies, 484 patients) (NS)
		  - neutropenia (2 studies, 200 patients) (NS)
		  - thrombocytopenia (3 studies, 381 patients) (NS)
		  - anaemia (2 studies, 192 patients) (NS)
		  - leukopenia (2 studies, 221 patients) (NS)
		- no difference in plasma concentrations of methotrexate (2 studies, 137 patients) (NS)
		
		1298CC compared to (1298AA + 1298AC):
		- decrease in the risk of leukopenia with OR = 0.51 (95% CI: 0.30-0.88) (2 studies, 221 patients) (S)
		- no difference in the risk of:
		  - hepatotoxicity (3 studies, 216 patients) (NS)
		  - myelosuppression (2 studies, 230 patients) (NS)
		  - thrombocytopaenia (3 studies, 375 patients) (NS)
		  - anaemia (2 studies, 186 patients) (NS)
			Authors' conclusion:
			"MTHFR, C677T and A1298C polymorphisms do not seem to be good markers of methotrexate-related toxicity in pediatric ALL."

ref. 13 - imm sup Owen SA et al. MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms. Pharmacogenomics J 2013;13:137-	3	Meta-analysis of 17 studies involving a total of 2,614 patients with rheumatoid arthritis. Ten studies involving a total of 1,375 patients containing data about effectiveness. 13 studies involving a total of 2,043 patients containing data about toxicity. Of the studies into effectiveness, seven studies were also included in the meta-analysis by Fan 2017, nine in the meta-analysis by Shao 2017, 3 in the meta-analysis by Qiu 2017 Sci Rep, all in the meta-analysis of Chen 2017, and two with a total of 325 patients in the meta-analysis by Morgan 2014 (Wessels 2006 and Lee 2009). Of the studies into toxicity, ten were also included in the meta-analysis by Huang 2020, seven in the meta-analysis by Fan 2017, twelve in the meta-analysis by Shao 2017, and 10 in the meta-analysis by Qiu 2017 Medicine, all in the meta-analysis of Chen 2017, and eight with a total of 1,355 patients in the meta-analysis by Song 2014 (van Ede 2001, Kumagai 2003, Berkun 2004, Aggarwal 2006, Kim 2006, Taniguchi 2007, Bohanec Grabar 2008 and Mena 2011). Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardi-	Authors' conclusion: "After combining our data with previous studies by meta-analysis, the random effects pooled odds ratios (OR) for both C677T and A1298C showed no association with efficacy or toxicity for either of the SNPs."

47. PubMed PMID: 21931346. **ref. 13, continuation**	(677CT + 677TT): AA (1298AC + 1298CC): AA	sed. Quality of the included studies was not analysed. There were no signs of publication bias. (677CT + 677TT) compared to 677CC: - no difference in the risk of non-response (10 studies, 1,375 patients) (NS). There was no heterogeneity between the studies. - no difference in the risk of toxicity (13 studies, 2,043 patients) (NS). There was major heterogeneity between the studies. The heterogeneity was reduced to a moderate level by both the removal of a study with deviating results (Kim 2006) and the limitation of the analysis to studies involving a Caucasian population. However, the increase in the risk of toxicity remained non-significant. A meta-regression analysis found no factors responsible for the heterogeneity. (A fixed effect model did find an increased risk of toxicity. However, this model may not be used in the case of heterogeneity between the studies.) (1298AC + 1298CC) compared to 1298AA: - no difference in the risk of non-response (8 studies, 1,140 patients) (NS). There was moderate heterogeneity between the studies. Limitation of the analysis to studies of Caucasian patients resulted in moderate, but non-significant heterogeneity. However, the difference in the risk of non-response remained non-significant. - no difference in the risk of toxicity (8 studies, 1,239 patients) (NS). There was moderate heterogeneity between the studies. Limitation of the analysis to studies of Caucasian patients resulted in moderate, but non-significant heterogeneity. However, the increase in the risk of toxicity remained non-significant. A meta-regression analysis found no factors responsible for the heterogeneity.	
ref. 14 - cytostat Yang L et al. Impact of methylenetetrahydrofolate reductase (MTHFR) polymorphisms on methotrexate-induced toxicities in acute lymphoblastic leukemia: a meta-analysis. Tumour Biol 2012;33:1445-54. PubMed PMID: 22528943.	3 677TT: E	Meta-analysis of 14 studies involving a total of 1,000 patients with acute lymphatic leukaemia. Of the 14 studies in this meta-analysis, 6 were also included in the meta-analysis by Yao 2019 and Zhu 2018, 8, with a total of 637 patients, in the meta-analysis by Lopez - Lopez 2013 (Kishi 2003, Pakakasama 2007, Huang 2008, Shimasaki 2008, Tantawy 2010, D'Angelo 2011, Karathanasis 2011 and Liu 2011) and 2, with a total of 145 patients, in the meta-analysis by Hagleitner 2014 (Chiusolo 2007 and Ongaro 2009). Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of absent or low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised. Quality of the included studies was not judged. There were no signs of publication bias in any of the comparisons. 677C>T: - the risk of hepatotoxicity (6 studies) was not elevated for TT versus CC (NS) or for CT versus CC (NS). The risk was elevated for (CT + TT) versus CC (OR = 1.70; 95% CI: 1.05-2.75) (S). There was heterogeneity between the studies for TT versus CC. - the risk of myelosuppression (3 studies) was elevated for TT versus CC (OR = 3.86; 95% CI: 1.53-9.75) (S). The risk was not elevated for CT versus CC (NS).	Authors' conclusion: "Results suggested that MTHFR C677T polymorphism was associated with significantly increased risk of methotrexate-induced toxicity, specifically liver toxicity, myelosuppression, oral mucositis, gastrointestinal toxicity, and skin toxicity. MTHFR A1298C polymorphism was found to be associated with decreased risk of skin toxicity."

ref. 14, continuation	677CT: AA (1298AC + 1298CC): AA[#]	- the risk of oral mucositis (6 studies) was elevated for TT versus CC (OR = 5.43; 95% CI: 2.38-12.39) (S). The risk was not elevated for CT versus CC (NS). - the risk of gastro-intestinal toxicity (four studies) was elevated for TT versus CC (OR = 6.94; 95% CI: 3.25-14.81) (S). The risk was not elevated for CT versus CC (NS). - the risk of skin toxicity (two studies) was not elevated for TT versus CC (NS), for CT versus CC (NS) or for (CT + TT) versus CC (NS). The risk was elevated for T allele versus the C allele (OR = 2.26; 95% CI: 1.07-4.74) (S). - there was no difference in the risk of haematological toxicity (3 studies), neurotoxicity (2 studies) and neutropenia (2 studies) (all three NS). 1298A>C: - the risk of skin toxicity (two studies) was reduced for (AC + CC) versus AA (OR = 0.11; 95% CI: 0.01-0.85) (S). The risk was not determined for CC versus AA and for AC versus AA. - there was no difference in the risk of hepatotoxicity, myelosuppression, oral mucositis, gastrointestinal toxicity, haematological toxicity, neurotoxicity, neutropenia and leukopenia (oral mucositis 3 studies, the rest 2 studies) (all eight NS)	
ref. 15 - cytostat/imm sup Spyridopoulou KP et al. Methylene tetrahydrofolate reductase gene polymorphisms and their association with methotrexate toxicity: a meta-analysis. Pharmacogenet Genomics 2012;22:117-33. PubMed PMID: 22143415.	3	Meta-analysis of 42 articles with 43 populations and therefore studies into methotrexate toxicity. The total number of patients in 34 studies was 3,948. No patient numbers were reported for 9 studies. The meta-analysis contained 8 of the 23 studies on 677C>T in the meta-analysis by Huang 2020, 2 of the 17 studies in the meta-analysis by Yao 2019, 3 of the 14 studies in the meta-analysis by Zhu 2018, 5 of the 18 studies on toxicity in the meta-analysis by Fan 2017, 11 of the 25 studies on toxicity in the meta-analysis by Shao 2017, 9 of the 21 studies in the meta-analysis by Qiu 2017 Medicine, 12 of the 27 studies in the meta-analysis of Chen 2017, 8 of the 12 studies in the meta-analysis by Song 2014 (a total of 1,488 patients), 3 of the 7 studies in the meta-analysis by Hagleitner 2014 (total of 605 patients), one of the 8 studies in the meta-analysis by Lopez-Lopez 2013 (with 26 patients), 10 of the 13 studies in the meta-analysis by Owen 2013 (with a total of 1,473 patients) and 5 of the 14 studies in the meta-analysis by Yang 2013 (with a total of 204 patients). ORs for homozygotes for the variant allele versus homozygotes for the wild-type allele and for heterozygotes versus homozygotes for the wild-type allele were calculated in two different ways: using logistical regression and using a multivariate model. All ORs were calculated using a random effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardised. Quality of the included studies was not analysed. There was no evidence for publication bias in any of the comparisons. 677TT compared to 677CC: - increase in the risk of more than two adverse events according to the logistical regression model (OR = 1.672; 95% CI: 1.009-2.769) (S) and a trend according to the multivariate model (OR = 1.670; 95% CI: 1.000-2.770) (NS) (8 studies). Ten studies found no increased risk for TT versus (CC + CT), but did find a trend. However, the risk was increased in the 7 studies in an Asian population group (OR = 1.655; 95% CI: 1.029-2.660) (S) and in the 7 studies with Hardy-Weinberg equilibrium for the patients without toxicity (OR = 1.604; 95% CI: 1.063-2.419) (S). For most of the ORs, there was no heterogeneity between the studies and there was no evidence of a publication bias.	Authors' conclusion: "These results indicate the association of MTHFR polymorphisms with methotrexate toxicity. However, further studies are needed to reveal the underlying biological mechanism of the association."

ref. 15, continuation	677TT: C 677TT: AA#	The study by Kim 2006 in patients with rheumatoid arthritis, which was included in both the comparison of TT versus CC and in the comparison of TT versus (CC + CT), had a major influence on the result. Exclusion of this study resulted in a vital decrease in the OR. The cumulative analysis revealed a decrease in the effect over time. - increase in the risk of hepatotoxicity according to the logistical regression model (OR = 4.191; 95% CI: 1.642-10.698) (S) and the multivariate model (OR = 4.260; 95% CI: 1.750-10,400) (S) (5 studies). Six studies found an increased risk for TT versus (CC + CT) (OR = 4.601; 95% CI: 2.139-9.896) (S). There was no evidence of heterogeneity between the studies or publication bias. - increased risk of neurotoxicity for TT versus (CC + CT) (OR = 3.398; 95% CI: 1.808-6.387) (2 studies) (S). There was no heterogeneity between the studies. - increase in the risk of toxicity in studies in which all patients received folate according to the logistical regression model (OR = 2.871; 95% CI: 1.107-7.450) (S) and the multivariate model (OR = 2.920; 95% CI: 1.110-7,770) (S) (6 studies). In 3 studies in which some of the patients received folate and in 11 studies in which no folate was given, there was no difference in the risk of toxicity (NS). In 7 studies in which all patients received folate, there was no difference for TT versus (CC + CT) (NS). - decrease in the risk of graft-versus-host reaction according to the logistical regression model (OR = 0.446; 95% CI: 0.242-0.821) (S) and the multivariate model (OR = 0.440; 95% CI: 0.240-0,820) (S) (2 studies). Four studies found a reduced risk for TT versus (CC + CT) (OR = 0.441; 95% CI: 0.253-0.771) (S). There was no heterogeneity between the studies and no evidence of a publication bias. - no difference in the risk of more than one adverse event according to the logistical regression model and the multivariate model (NS) (20 studies), but there was a trend for an increased risk. A total of 25 studies found no increased risk for TT versus (CC + CT). However, the risk was elevated in the eighteen studies with Hardy-Weinberg equilibrium for the patients without toxicity (OR = 1.575; 95% CI: 1.100-2.254) (S). There was no heterogeneity between the studies for this comparison. There was no evidence of a publication bias. The cumulative analysis revealed a small decrease in the effect over time, but the effect became stable after 2006. - no difference in the risk of haematological toxicity according to the logistical regression model and the multivariate model, but there was a trend for an increased risk (NS) (3 studies). In 5 studies, there was no difference for TT versus (CC + CT) (NS). However, the risk was elevated in the 3 studies with Hardy-Weinberg equilibrium for the patients without toxicity (OR = 2.551; 95% CI: 1.055-6.169) (S). There was heterogeneity between the studies for some ORs. There was no evidence of a publication bias. - no difference in the risk of gastrointestinal adverse events (NS) - no difference in the risk of mucositis according to the logistical regression model and the multivariate model (4 studies) (NS). In 5 studies, there was no difference for TT versus (CC + CT) (NS). - no difference in the risk of an adverse event in patients with rheumatoid arthritis according to the logistical regression model and the multivariate model (NS) (6 studies), but there was a trend for an increased risk. Seven studies found no increased risk for TT versus (CC + CT).	

ref. 15, continuation	677CT: C	However, the risk was elevated in the 4 studies with Hardy-Weinberg equilibrium for the patients without toxicity (OR = 1.735; 95% CI: 1.129-2.666) (S). There was no heterogeneity between the studies for the abovementioned comparison. There was no publication bias. - no difference in the risk of an adverse event in patients with haematological conditions according to the logistical regression model and the multivariate model (NS) (7 studies). Ten studies found no increased risk for TT versus (CC + CT). - decrease in the risk of an adverse event in patients following haematopoietic stem cell transplantation according to the logistical regression model (OR = 0.413; 95% CI: 0.226-0.753) (S) and the multivariate model (OR = 0.410; 95% CI: 0.220-0,750) (S) (3 studies). Five studies found a reduced risk for TT versus (CC + CT) (OR = 0.435; 95% CI: 0.252-0.754) (S). There was no heterogeneity between the studies. 677CT compared to 677CC: - no difference in the risk of more than two adverse events according to the logistical regression model and the multivariate model (NS) (8 studies). Also for (CT+TT) versus CC, no difference was found (14 studies) (NS). There was strong heterogeneity between the studies for this comparison. - no difference in the risk of hepatotoxicity according to the logistical regression model and the multivariate model (NS) (5 studies). Nine studies found an increased risk for (CT+TT) versus CC (OR = 2.170; 95% CI: 1.305-3.608) (S). There was no evidence of heterogeneity between the studies or publication bias. - no difference in the risk of neurotoxicity for (CT+TT) versus CC (4 studies) (NS). There was no heterogeneity between the studies. - increase in the risk of toxicity in studies in which all patients received folate according to the logistical regression model (OR = 2.736; 95% CI: 1.347-5.559) (S) and the multivariate model (OR = 2.820; 95% CI: 1.320-6,000) (S) (6 studies). In 3 studies in which some of the patients received folate and in 11 studies in which no folate was given, there was no difference in the risk of toxicity (NS). In 7 studies in which all patients received folate, there was no difference for (CT+TT) versus CC (NS). - no difference in the risk of graft-versus-host reaction according to the logistical regression model and the multivariate model (2 studies) (NS). In 4 studies, there was no difference for (CT+TT) versus CC (NS). - no difference in the risk of more than one adverse event according to the logistical regression model and the multivariate model (NS) (20 studies). In 32 studies, no increased risk was found for (CT+TT) versus CC, but there was a trend (NS). There was heterogeneity between the studies for this comparison. The cumulative analysis revealed a small decrease in the effect over time, but the effect became stable after 2006. - no difference in the risk of haematological toxicity according to the logistical regression model and the multivariate model (NS) (3 studies). In 6 studies, there was no difference for (CT+TT) versus CC (NS). There was no heterogeneity between the studies for this comparison. - no difference in the risk of gastrointestinal adverse events (NS)	

ref. 15, continuation		- no difference in the risk of mucositis according to the logistical regression model and the multivariate model (4 studies) (NS). In 5 studies, there was no difference for (CT+TT) versus CC (NS). - no difference in the risk of an adverse event in patients with rheumatoid arthritis according to the logistical regression model and the multivariate model (NS) (6 studies). In 13 studies, no difference in risk was found for (CT+TT) versus CC. There was strong heterogeneity between the studies for this comparison. - no difference in the risk of an adverse event in patients with haematological conditions according to the logistical regression model and the multivariate model (NS) (7 studies). In 9 studies, no difference in risk was found for (CT+TT) versus CC. - no difference in the risk of an adverse event in patients following haematopoietic stem cell transplantation according to the logistical regression model and the multivariate model (NS) (3 studies). In 5 studies, no difference in risk was found for (CT+TT) versus CC.	
	1298C C: C	1298CC compared to 1298AA: - increase in the risk of toxicity in 4 studies in which no folate was given, according to the logistical regression model (OR = 4.131; 95% CI: 1.864-9.155) (S) and the multivariate model (OR = 3.830; 95% CI: 1.950-7.490) (S). In 4 studies in which some of the patients received folate and in one study in which no folate was given, there was no difference in the risk of toxicity (NS). In 6 studies in which no folate was given, there was no difference for CC versus (AA+AC) (NS). There was strong heterogeneity between the studies for this comparison. - increase in the risk of more than one adverse event according to the logistical regression model (OR = 1.690; 95% CI: 1.011-2.825) (S), but no difference according to the multivariate model (NS) (9 studies). No difference in the risk of more than one adverse event for CC versus (AA+AC) (NS) (12 studies). - no difference in the risk of an adverse event in patients with rheumatoid arthritis according to the logistical regression model and the multivariate model (NS) (3 studies). Four studies found a reduced risk for CC versus (AA+AC) (OR = 0.508 (95% CI: 0.273-0.936) (S). There was no heterogeneity between the studies for this comparison. - no difference in the risk of more than two adverse events according to the logistical regression model and the multivariate model (NS) (5 studies). No difference in the risk of more than two adverse events for CC versus (AA+AC) (NS) (7 studies).	
	1298A C: AA	1298AC compared to 1298AA: - no difference in the risk of toxicity in 4 studies in which no folate was given, according to the logistical regression model and the multivariate model (NS). In 4 studies in which some of the patients received folate, there was also no difference in the risk of toxicity (NS). In 5 studies in which no folate was given, there was an increase in the risk of toxicity for (AC+CC) versus AA (OR = 1.926; 95% CI: 1.098-3.379) (S). There was no heterogeneity between the studies for this comparison. In one study in which all patients were given folinic acid, there was also an increase in the risk of toxicity for (AC+CC) versus AA (OR = 2.425; 95% CI: 1.289-4.560) (S). In 6 studies in which some of the patients received folate, there was no difference (NS). There was heterogeneity between the studies for	

ref. 15, continuation		this comparison. - increase in the risk of more than one adverse event according to the logistical regression model (OR = 1.889; 95% CI: 1.232-2.896) (S), but no difference according to the multivariate model (NS) (9 studies). No difference in the risk of more than one adverse event for (AC+CC) versus AA (NS) (12 studies). - no difference in the risk of an adverse event in patients with rheumatoid arthritis according to the logistical regression model and the multivariate model (NS) (3 studies). In 5 studies, no difference in risk was found for (AC+C) versus AA (NS). There was heterogeneity between the studies for this comparison. - no difference in the risk of more than two adverse events according to the logistical regression model and the multivariate model (NS) (5 studies). No difference in the risk of more than two adverse events for (AC+CC) versus AA (NS) (7 studies).	
ref. 16 - imm sup Fisher MC et al. Meta-analysis of methylene-tetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. J Rheumatol 2009;36:539-45. PubMed PMID: 19208607.	3 (677CT + 677TT): AA (1298AC + 1298CC): AA	Meta-analysis of 8 studies into methotrexate toxicity involving a total of 1,441 patients with rheumatoid arthritis. All 8 studies were also included in the meta-analyses by Spyridopoulou 2012 and Owen 2013. Of the 8 studies, five were also included in the meta-analysis by Fan 2017, all eight in the meta-analyses by Shao 2017 and Chen 2017, one with a total of 200 patients in the meta-analysis by Morgan 2014 (Wessels 2006) and 7 studies with a total of 1,335 patients in the meta-analyses by Song 2014, Qiu 2017 Medicine, and Huang 2020 (van Ede 2001, Kumagai 2003, Berkun 2004, Aggarwal 2006, Kim 2006, Wessels 2006 and Taniguchi 2007). The studies in the meta-analysis differed in the definition of toxicity, the methotrexate dose and the use of folate. There were also differences in ethnicity between the studies and the frequency of the variant allele differed between the different ethnicities. This means that the percentage of homozygotes for the variant allele also differed in the group of patients with this allele. Meta-analyses were performed with both a random-effects and a fixed-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent, but the method of data exaction was not mentioned. Quality of the included studies and possible publication bias were not analysed. (677CT + 677TT) compared to 677CC: - increase in the risk of toxicity when calculated according to a fixed effects model (OR = 1.71; 95% CI: 1.32-2.21) (S), but no significant difference when calculated according to a random effects model (NS) (1298AC + 1298CC) compared to 1298AA (5 studies, 618 patients): - no difference in the risk of toxicity (NS)	Authors' conclusion: "In a fixed effects model, the C677T polymorphism was associated with increased toxicity. The A1298C polymorphism was not associated with increased toxicity."

Risk group	Combination therapy with methotrexate and fluorouracil. The effect of fluorouracil is possibly affected by MTHFR gene variations.

Comments:

- Only articles investigating the effect of at least one of the MTHFR gene variants in more than 800 methotrexate users were included. In the case of a meta-analysis for which an update was published before 2015, only the update was included. Other articles did not contribute sufficiently to the burden of proof.

- Cost-effectiveness:
  - Plumpton CO et al. A systematic review of economic evaluations of pharmacogenetic testing for prevention of adverse drug reactions. Pharmacoeconomics 2016;34:771-93. PubMed PMID: 26984520.
    The authors performed a systematic literature review of economic evaluations of pharmacogenetic tests of MTHFR prior to prescription of methotrexate. The authors conclude that economic evidence was inconclusive with respect to genotyping of MTHFR prior to methotrexate, due to the presence of only one study.
    A single, high-quality study evaluating testing for the MTHFR C677T gene variant prior to methotrexate treatment in rheumatoid arthritis patients found therapy with prior genotyping to be both better (resulting in more quality adjusted life-years) and cheaper than therapy without prior genotyping (Kim SK et al. Cost-effectiveness analysis of MTHFR polymorphism screening by polymerase chain reaction in Korean patients with rheumatoid arthritis receiving methotrexate. J Rheumatol 2006;33:1266-74), although there are no current FDA or EMA recommendations for testing. In the model of Kim 2006, more patients continued methotrexate with prior MTHFR 677C>T genotyping (95.58%) than without (94.03%).

Date of literature search: 11 November 2020.

	Genotype	**Code**	**Gene-drug interaction**	**Action**	**Date**
KNMP Pharmacogenetics Working Group decision	677TT	4E	No	No	7 June 2021
	677CT	4C	No	No	

Mechanism:
Methotrexate inhibits dihydrofolate reductase, which converts dihydrofolate to tetrahydrofolate. Tetrahydrofolate is required for the synthesis of purine nucleotides and following conversion to 5,10-methylenetetrahydrofolate, it is also required for the synthesis of thymidine nucleotides by thymidylate synthase. Methotrexate also inhibits thymidylate synthase directly. The toxicity of methotrexate can be reduced by administration of the tetrahydrofolate precursors folic acid or leucovorin (folinic acid).
The enzyme MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which can in turn be converted to tetrahydrofolate. Reduced activity of the enzyme MTHFR results in decreased intracellular tetrahydrofolate concentrations.
Therefore, both methotrexate and MTHFR affect folic acid metabolism and the intracellular concentrations of tetrahydrofolate. For this reason, gene variants that result in reduced MTHFR enzyme activity could influence the effectiveness and toxicity of methotrexate.