Pemetrexed is a newer antifolate drug that has been approved as first-line treatment for patients with advanced non-squamous, non-small cell lung cancer (NSCLC) in combination with cisplatin, and as single agent for relapsed or chemotherapy refractory NSCLC after platinum-containing chemotherapy, at a dose of 500 mg/m(2). Pemetrexed undergoes intracellular activation by poly-gamma-glutamylation, that is essential for its antiproliferative activity. Polyglutamate derivatives mainly inhibit three key enzymes of intracellular folate metabolism, i.e. thymidylates synthase (TYMS), dihydrofolate reductase (DHFR), and glycaminide ribonucleotide formyltransferase (GARFT), with TYMS being the most relevant target. Pemetrexed undergoes rapid renal elimination as unchanged parent compound, with a terminal half-life of between two to five hours. In later clinical development, the usefulness of supplementation with folic acid and vitamin B(12) became evident, to control pemetrexed-related toxicity. The results from the phase III upfront registration study, a retrospective observational data, and a recent maintenance study of pemetrexed in NSCLC suggest histological subtype to be the most important predictive marker for clinical outcome in patients receiving pemetrexed, Pemetrexed is active in patients with non-squamous cell NSCLC while no benefit is seen in patients with squamous-cell histology, possibly as a result of different expression of intratumoral TYMS. These are important steps towards individualisation of anticancer treatment in patients with advanced NSCLC.